Integrins as therapeutic targets: lessons and opportunities.

AUTHOR(S)

Dermot Cox, Marian Brennan, Niamh Moran

Integrins as therapeutic targets: lessons and opportunities

Dermot Cox BSc, PhD, Marian Brennan BSc, PhD and Niamh Moran BSc, PhD

Molecular and Cellular Therapeutics, Royal College of Surgeons in Ireland, Dublin.

Abstract | The integrins are a large family of cell adhesion molecules that are essential for the regulation of cell growth and function. The identification of key roles for integrins in a diverse range of diseases, including cancer, infection, thrombosis and autoimmune disorders has revealed their substantial potential as therapeutic targets. However, so far, pharmacological inhibitors for only three integrins have received approval. This article discusses the structure and function of integrins, their physiological functions and roles in disease and the checkered history of the approved integrin antagonists. Recent advances in the understanding of integrin function, ligand interaction and signalling pathways suggest novel strategies for inhibiting integrin function that could help harness their full potential as therapeutic targets.

Introduction

The first integrin was identified in 1986, when a complex, fibronectin-binding, membrane glycoprotein that was integral in linking the cytoskeleton with the extracellular matrix was identified[1]. Following this initial discovery, it soon became clear that this glycoprotein was only one member of a diverse family of receptors exhibiting similar functions, all of which are heterodimers composed of an α and a β subunit (FIG. 1)[2]. Today, the integrins are known to have vital roles in both health and disease and their potential to be therapeutically targeted is now widely recognized.

So far, pharmacological inhibitors have been approved for three integrins, although each of these classes of inhibitors has important limitations. The first integrin to be successfully targeted was the platelet integrin $\alpha_{IIb}\beta_3$ which plays a key role in platelet

aggregation. In the 1990's three $\alpha_{IIb}\beta_3$ inhibitors —the antibody fragment abciximab and the small molecule inhibitors eptifibatide and tirofiban, all of which are administered intravenously —were approved to reduce the risk of ischaemic events in patients with acute coronary syndromes (ACS) and those undergoing percutaneous coronary intervention (PCI)[3]. However, despite initial expectations that antagonists targeting this integrin would be blockbuster drugs, attempts to develop oral antagonists for more convenient administration were not successful and the use of the approved intravenous inhibitors has largely been restricted to high-risk patients. Instead, clopidogrel, an orally active ADP-receptor antagonist, filled the market that was expected for $\alpha_{IIb}\beta_3$ antagonists and became the second biggest-selling drug globally[4].

Antagonists have also been approved for two integrins involved in autoimmune disorders. Natalizumab, a monoclonal antibody (mAb) that binds to the α_4 integrin subunit, is approved for the treatment of multiple sclerosis[5] and Crohn's disease[6] and efalizumab, a mAb that targets the $\alpha_L\beta_2$ integrin, was approved for the treatment of moderate to severe psoriasis[7]. However, both of these agents are associated with progressive multifocal leukoencephalopathy (PML) a potentially fatal side effect thought to be related to their immune suppressant properties[8]. The initial reports of PML cases led to withdrawal from the market of natalizumab in 2005, but supported by its high efficacy in reducing the rate of relapses in multiple sclerosis and the level of medical need in this indication, it was reintroduced with a black-box label warning and a risk-management strategy in 2006. However, efalizumab was simply withdrawn from the market in 2009.

Recent advances in our understanding of the nature of ligand-integrin interactions, integrin structure and signalling have created opportunities for the development of different types of integrin antagonists, as well as raising the possibility of targeting down-stream signalling events as an alternative to blocking receptor occupancy. This article discusses how such advances could inform potential future strategies for the development of integrin targeted drugs, with a focus on those integrins for which therapeutic inhibitors have been developed. Issues associated with the clinical use of approved integrin inhibitors are also considered.

Physiological role of integrins

Integrins, which are found on nearly all cells, mediate cell-cell and cell-substrate interactions. This extensive family of cell-adhesion molecules, which includes more than twenty known α-β heterodimer combinations (FIG. 1), bind to a diverse collection of ligands which are mostly large molecules, usually found in the sub-endothelial matrix, including fibronectin[1], vitronectin[9] and collagen[10]. Other ligands are plasma proteins including complement factors[11], C-reactive protein[12] and fibrinogen[13]. Many integrins also recognize the amino acid sequence Arg-Gly-Asp (RGD) in their ligands[2,14].

However, integrins do not simply act as "glue-like" molecules; they are true receptors, generating intracellular signals[15]. Given that contact with other cells and extra-cellular matrix components regulates the activity of all cells, and that integrins are an important family of receptors that mediate these interactions, they have essential roles in the function of most cells. Areas in which integrins are especially important are those that involve growth of tissue or where cell attachment is necessary for function. Thus, embryonic development[16] and angiogenesis[17] are critically dependent on

integrins, as is the immune system[18] in which immune cell attachment is necessary for normal function (see below).

Integrins and disease

Although integrins are known to be involved in a variety of disorders, identifying the specific integrin involved and their precise role is difficult because many diseases are multi-factorial and integrins are only one of many types of receptors involved. Moreover, many cells posses multiple integrins that exhibit complementary binding properties. In this section, a brief overview of the disorders in which integrins are known to have an important role is presented, together with discussion of clinical experience with relevant integrin-targeted drugs where applicable

Thrombosis. The first disease-related process to be clearly associated with integrins was thrombosis, the formation of a blood clot inside a blood vessel, which occurs when platelets adhere to damaged blood vessels and become activated[19]. These activated platelets recruit other platelets, resulting in the formation of a haemostatic plug. This is an essential mechanism for preventing blood loss, but inappropriate thrombus formation can lead to a stroke or heart attack.

In the 1970s, it was noted that some patients with a severe bleeding disorder known as Glanzmann's thrombasthenia lacked two functional glycoproteins (GPIIb and GPIIIa) on their blood platelets, which are now known as the integrin $\alpha_{IIb}\beta_3$[20]. It was subsequently found that the platelet-platelet interaction that mediates thrombus formation is facilitated by fibrinogen binding to the platelet-specific integrin $\alpha_{IIb}\beta_3$ following platelet activation caused by thrombotic stimuli[13].

This knowledge provided the basis for the development of the first three integrin-targeted drugs to be approved which were introduced in the 1990's[21]. The first anti-integrin to be commercialized was the anti-β_3 monoclonal antibody abciximab (ReoPro; Centocor/Eli Lilly). It is a potent inhibitor of platelet aggregation and has been extensively tested in clinical studies[22-23] resulting in approval for use during percutaneous coronary intervention (PCI). Eptifibatide (Integrilin; Millenium/Schering-Plough) is a cyclic-peptide derived from barbourin, a component of viper venom. Like barbourin it contains a KGD sequence rather than an RGD sequence. It is very potent and highly effective at inhibiting $\alpha_{IIb}\beta_3$[24] and is currently used for patients undergoing PCI[25-26] and in patients with acute coronary syndromes[27]. Tirofiban (Aggrastat; Merck) is a non-peptide, small-molecule inhibitor of $\alpha_{IIb}\beta_3$, although it has no oral activity (see Table 1). Like eptifibatide its development was based on a viper venom peptide (echistatin). It was approved for use in PCI and acute coronary syndromes[28-29].

Inspired by the success of these intravenous $\alpha_{IIb}\beta_3$ antagonists, the development of orally active antagonists of $\alpha_{IIb}\beta_3$ was an area of intense activity in the 1990's. Several compounds went into phase III trials including xemilofiban, orbofiban, sibrafiban and lotrafiban (see table 1). However, in contrast to the success of the intravenous compounds, the oral compounds showed no benefit and patients even did worse due to an increase in cardiovascular events[30]. This surprising failure of the oral $\alpha_{IIb}\beta_3$ antagonists was probably not due to any single factor[31]. Although there were problems with agonist-like activity with some of these drugs, other factors such as

poor bioavailability and under-dosing also played a role. There was also a lack of understanding of the role of $\alpha_{IIb}\beta_3$ in thrombosis, in particular its role in signalling.

Immune system disorders. Integrins have a key role in the migration and interaction of cells of the immune system. Both β_1 and β_2 integrins are important in immune function where they play essential roles in localizing the immune response to the site of inflammation[11] and a defect in β_2 integrins leads to life-threatening immune dysfunction (leucocyte adhesion deficiency, LAD)[32]. Engagement of the T-cell receptor and subsequent inside-out signalling leads to activation of T-cell integrins[18].

The initial focus on anti-integrins for immune disorders was on targeting $\alpha_L\beta_2$ and α_4 integrins. Interest in developing anti-α_4 integrins was catalysed by the discovery in the early 1990s that $\alpha_4\beta_1$ integrin had a key role in the migration of lymphocytes to inflamed regions of central nervous system in rodent models of multiple sclerosis, and that blockade of α_4 with a monoclonal antibody could inhibit the development of paralysis in these models[33]. Natalizumab, a humanized mAb specific for the α_4 integrin subunit was found to be effective in the treatment of multiple sclerosis[5,34-39] and also for the inflammatory bowel disorder Crohn's disease[6]. Inhibitors of α_4 integrins have been effective in animal models of asthma but none have been effective in Phase II trials[40].

Natalizumab (Tysabri; Elan/Biogen-Idec) was first approved by the US FDA for the treatment of multiple sclerosis in 2004, and has shown substantial efficacy in clinical trials[37-39]. However, a few months after its initial approval, several patients treated with natalizumab developed a fatal, progressive multifocal leukoencephalopathy

(PML)[41], leading the manufacturer to withdraw the drug from the market. It was found that PML in the initial cases was due to re-activation of JC virus infection of the central nervous system[42]. However, this might not be specific to JC virus, as herpes virus 6 re-activation has also been seen in natalizumab-treated patients[43]. Reflecting the major need for effective treatments for multiple sclerosis, natalizumab was reintroduced in to the market in 2006 with a black-box warning, and a risk evaluation and mitigation strategy (REMS) known as TOUCH to identify patients at risk of developing PML. Annual sales of the drug surpassed $US 1 billion in 2009, but it remains under close regulatory scrutiny.

Antagonists of $\alpha_L\beta_2$ integrin (also known as leukocyte-function-associated antigen 1, LFA1), which is involved in the activation and migration of T cells, have shown benefit in the inflammatory skin disorder psoriasis[44-45]. Efalizumab (Raptiva; Genentech), a humanized mAb specific for the α_L subunit, was approved in 2003 for the treatment of plaque psoriasis and shown to be effective in long-term trials[46-49]. However, reports of cases of PML associated with efalizumab led to its withdrawal from the market in 2009[50].

Cancer. During carcinogenesis, the growth of the tumour and its subsequent metastasis is highly dependent on tumour cells being able to regulate their attachment to the extracellular matrix and adjacent cells. Given that integrins play important roles in cell attachment, survival, migration, invasion and angiogenesis[17,51-53], which are all critical for carcinogenesis, they have attracted considerable attention as potential anti-cancer targets. Usually anti-integrins are used in combination with other anti-cancer agents but they also have potential as imaging agents in cancer diagnosis[54-55].

Many different integrins have been implicated in carcinogenesis. One of the most important is $\alpha_V\beta_3$[56] which is known to act as a trigger for cell survival[57] and this may be important in angiogenesis where endothelial cell survival is critical. It also plays a key role in metastasis where its expression in melanoma is associated with highly metastatic disease[58]. Etaracizumab is a monoclonal antibody to $\alpha_V\beta_3$ that is undergoing early clinical development for treatment of melanoma[59-60] and solid tumours[61]. The cyclic-RGD peptide cilengitide is a specific $\alpha_V\beta_3$ antagonist and is currently under going clinical trials for use in glioblastoma[62-63] and in other brain cancers[64] and has recently become the first anti-integrin to enter Phase III trials for cancer[65]. The orally-active, non-peptide $\alpha_V\beta_3$ antagonist MK 0429 is under going clinical development for prostate cancer[66].

Another integrin of interest in cancer is $\alpha_5\beta_1$ which is involved in cell survival and migration[67] and angiogenesis[68]. Volociximab is an anti-α_5 antibody under development for solid tumours[69-70]. Recent evidence suggests that inhibition of both $\alpha_V\beta_3$ and $\alpha_5\beta_1$ may be required for optimum effects on angiogenesis[71].

Infection. A number of infectious agents have developed the ability to interact with integrins and subsequently become internalized allowing access to the intracellular milieu[72-73]. Three general mechanisms are used to achieve this: first the binding of integrin ligands that mediate an interaction with an integrin, for example the binding of *Staphylococcus aureus* and *Staphylococcus epidermidis* to fibrinogen[74-75] and *S. aureus* to fibronectin[76] mediate an interaction with platelet $\alpha_{IIb}\beta_3$ triggering platelet aggregation. The second mechanism is direct interaction with an integrin for example *Papilloma virus* binds to $\alpha_6\beta_4$[77], *Rotavirus* binds to $\alpha_2\beta_1$ and $\alpha_4\beta_1$[78-80] and Ebola

glycoprotein interacts with $\alpha_5\beta_1$[81]. The third mechanism is binding of bacterial secreted products to an integrin, for example $\alpha_L\beta_2$ binds RTX (repeat in toxin) family of cytotoxins[82-84] and *Helicobacter pylori* Vac A toxin binds to β_2 integrins[85]. *H. pylori* CagL[86], *S. aureus* α-toxin[87] and *Bacillus anthracis* lethal factor-protective antigen complex[88] interact with $\alpha_5\beta_1$. Integrins can also modulate the immune response to infection; for example mindin, an $\alpha_M\beta_2$ and $\alpha_4\beta_1$ ligand[89], acts as a pattern recognition molecule for microbes[90], C-reactive protein binds to $\alpha_{IIb}\beta_3$[12] and $\alpha_M\beta_2$ and $\alpha_X\beta_2$ are complement receptors[11]. However, the role of integrins in infection has yet to be exploited for therapeutic purposes but inhibition of these interactions, which are involved in the infection process, has a potential role in the treatment of infection.

Osteoporosis. Osteoporosis occurs when the balance between bone formation and degradation is disturbed. Integrins play an important role in the function of osteoclasts, which mediate bone resorption. Osteoclast $\alpha_1\beta_1$ is responsible for adhesion of osteoclasts to collagen, and polymorphisms in this receptor are related to bone mineral density and fractures[91]. $\alpha_V\beta_3$ is also important in osteoclast function and polymorphisms in this receptor are also associated with increased rate of fracture[92]. An antagonist of this receptor has been shown to increase bone density in post-menopausal women in a Phase II study[93]. However, there is no evidence that this or any other anti-integrin is undergoing clinical development for osteoporosis.

Emerging opportunities. The initial drug discovery work for several of the integrins described above often occured in the absence of detailed understanding of their function. However, recent advances in our knowledge of the structure of integrins, their interaction with ligands and their signalling pathways has shed light on the

difficulties associated with early integrin antagonists and suggests novel strategies for more effectively inhibiting integrin function[94-95]. The remainder of the article first discusses relevant advances in structural understanding and integrin signalling, and then considers how these could be applied to the development of novel and/or improved integrin-targeted drugs.

Insights into integrin structure

Integrin domain structure. Advances in crystallography, together with mutagenesis studies, have yielded a great deal of information regarding integrin structure. A schematic of the integrin domain structure is outlined in FIG. 2a. The α-chain is made up of a short cytoplasmic domain and a transmembrane domain attached to two calf domains that make up the lower leg region. The thigh domain makes up the upper leg region, which is followed by the 7 repeats constituting the β-propeller. The interactive domain (I-domain) is inserted between repeats 2 and 3 in nine of the α subunits (α_1, α_2, α_{10}, α_{11}, α_L, α_D, α_M, α_X, α_E) and plays a role in ligand binding. This is known as the α-I domain.

The β-chain has a longer cytoplasmic domain, a transmembrane domain, four integrin epidermal growth factor-like domains making up the lower leg region and an I-like domain inserted into the hybrid domain forming the head domain. This I-like domain is known as the β-I domain. The N-terminal hybrid domain and the plexin semaphorin integrin (PSI) domain fold over to form the upper leg domain, exposing the β-I domain.

Integrin conformations. Integrins display three distinct conformations, a resting inverted V shaped conformation, an intermediate partially activated extended state, and a fully activated open, ligand-binding conformation (FIG. 2b)[96-98]. The PSI and hybrid domains swing out away from the α-chain in the transition between intermediate and fully activated states[97]. Some integrins can bind their ligands in a resting state while others require activation prior to binding. Activation is mediated by signalling events leading to alterations in the intracellular domains, also known as inside-out signalling. Ligand binding results in transmission of a signal into the cell which is known as outside-in signalling (FIG 2c). A number of partial intermediates between these three states have also been proposed[97,99-100]. Crystal structure data for the resting $\alpha_v\beta_3$ and activated $\alpha_{IIb}\beta_3$ has been assimilated with electron microscopy data providing a model of the intermediate structures[99-101].

Ligand-binding. Both the α-I and β-I domains contain a metal ion that interacts with the ligand, known as the metal-ion-dependent adhesion site (MIDAS) (FIG. 3a and B). If the α-I domain is present, it forms the ligand-binding domain. If the α-I domain is absent, the ligand-binding domain lies between the two subunits, between the β-I domain (on the β-subunit) and the β-propellor domain (on the α-subunit) (FIG. 3b). The α-I and β-I domains both contain a central Rossman fold surrounded by seven helices (see FIG. 3a).

Ligand binding leads to allosteric changes within the molecule through movements in the α7 and α1 helices in the α-I and β-I domains (FIG. 3c)[102]. Movement of these helices is thought to be responsible for the shape changes in the ectodomain upon

ligand binding. The piston-like displacement of the α7 helix leads to changes in the legs and ultimately tail separation, likely translating changes further into the cytoplasmic regions leading to signalling events (FIG. 2c, upper schematic). This is known as outside-in signalling.

The structural changes resulting from inside-out signalling are thought to be due to separation of the lower legs of the ectodomain due to changes in the cytoplasmic regions which are then translated to the head region, ultimately leading to changes in the ligand binding domain, which increases affinity for the integrins' ligand, (FIG. 2c, lower schematic)[97]. Due to the dynamic changes upon activation, there are a number of different regions to be considered when investigating integrins as drug targets.

Integrin signalling

Integrins were originally thought to simply be adhesion molecules and not true receptors as they had no homology to the four main types of known receptors and no evidence of intracellular signalling motifs. However, recent work has clearly identified signalling molecules that associate with integrins. Many studies have focused on the short cytoplasmic tails and the transmembrane domains of the integrin α and β subunits (Box 1, panel a), which may have applications in future anti-integrin strategies[103-105].

Role of integrin cytoplasmic tails. Integrin activation occurs as a result of inside-out signalling, whereby cell-activating stimuli initiate a cascade of intracellular events. These events ultimately impact on the integrin cytoplasmic tails (CTs) and cause a

conformational change in the extracellular portion of the integrin[13]. Over two decades of research has expanded our understanding of the intracellular signalling pathways that affect the integrin cytoplasmic tails, resulting in integrin activation[94,105]. However, the intracellular consequences of integrin activation, ligand occupancy and the ensuing outside-in cascade of cell-signalling events remains unclear in many cellular systems[106].

The transmembrane helices and short cytoplasmic domains of integrin α-and β-subunits are critical in coordinating these cellular responses[94]. Integrin cytoplasmic tails are between 15 and 78 amino acids in length for α-subunits and between 46 and 68 amino-acids in length for β-subunits (with the exception of the atypical β_4 subunit). They contain no known enzymatic activity and must therefore interact with cytoplasmic signalling or adaptor proteins to affect or respond to the changing cellular activation state.

Strong evolutionary conservation of membrane-proximal sequences KxGFFKR and LLxxxHDRRE (Box 1, panels b and c) in the α and β subunits respectively, suggest that these sequences hold a key to understanding the coordination of molecular events that either cause, or result from, integrin activation. The integrin α- and β membrane-proximal domains interact weakly, but distinctly, constraining the integrin in a 'resting' conformation[107-108]. An intracellular molecular clasp between oppositely charged membrane-adjacent residues strengthens this conformational state[94]. Events that are triggered by cellular activation can interrupt this association, allowing the binding of alternative cytoplasmic binding partners to each individual α or β-CT, driving integrin activation and downstream signalling events[109]. Integrin β-CTs also contain one or two highly conserved NPXY/F domains at their membrane-distal C-

terminals, suggestive of a regulatory role in recruiting phosphotyrosine–binding signaling proteins. These phosphotyrosine-like motifs act as a docking site for talin[110], a cytoskeletal molecule that plays a key role in integrin-mediated cell adhesion and cytoskeletal reorganization. Competition by proteins such as filamin and tensin for binding to this site in its phosphorylated or non-phosphorylated state underlies our current understanding of the cellular regulation of integrin activation[105].

β-integrin tails. For the β-integrin CTs, talin is the major interacting cytoplasmic protein[94]. Its binding to the membrane-distal section of the β-tail is a common and necessary event in integrin activation in many cell types and events including T-cell adhesion and migration on ICAM-1[111], lymphocyte-endothelial cell interactions[112], macrophage-mediated phagocytosis by $\alpha_M\beta_2$ integrin[113], $\alpha_{IIb}\beta_3$–mediated platelet adhesion and spreading[110] and clathrin-mediated endocytosis in the neuronal synapse[114]. Whole-animal disruption of talin expression is lethal in embryonic mice[115] but conditional inhibition of talin expression demonstrates that talin is required specifically for the outside-in activation of integrins *in vivo*[116].

Talin. Talin (270 kDa) comprises a globular N-terminal head region (~50kDa) and an extended flexible rod domain (~220kDa). The head contains an N-terminal FERM (band 4.1, ezrin, radixin, and moesin) domain with three subdomains: F1, F2, and F3 which comprise the binding sites for the β-integrin CTs as well as for filamentous actin (F-actin)[117]. The talin rod contains additional binding sites for other integrin domains, for actin and several binding sites for vinculin, which itself has multiple partners. The talin rod domain can also bind to the talin head-domain in an anti-

parallel manner, masking the integrin-binding regions and exerting an auto-inhibitory function. This is an important regulatory mechanism for preventing inappropriate integrin activation[118].

Talin binding to integrin β-tails is highly regulated[94]. Kindlin[119], ILK[120] and tensin[105] are just three proteins known to regulate talin binding to integrin β-cytoplasmic tails. Tight coordination of the choreography of protein interactions appears to underlie the control of downstream integrin signalling and adhesion events.

α-integrin tails. The role for the α-integrin CTs in the regulation of integrin functions in cells remains largely an unanswered question. It is widely believed that the α-integrins regulate the affinity of the integrin complex for its ligand[121-122] through a coordinated association between the highly-conserved KxGFFKR α-integrin signature motif and the membrane-proximal region of the integrin β- CT[108-109,123]. Dissociation of the integrin α-β tails results in integrin activation and an enhanced affinity for ligand binding[122,124]. Moreover, deletion of the KxGFFKR regulatory region, or introduction of mutations, induces a constitutive activation of the integrin[122,125]. Thus the integrin α-cytoplasmic tail exerts a regulatory influence on integrin activation by controlling the availability of the β-cytoplasmic tail for binding to talin. Following activation-induced binding of talin, the α-KxGFFKR motif is liberated from its β-tethered position and it is free to interact with its own range of adaptor proteins[126]. However, available evidence suggests that the affinity of α-CTs for β-CTs is not very strong[107,127], suggesting that other facilitatory cytoplasmic proteins might enhance this function. Many such proteins have been identified, but the choreography of their interaction is not fully understood[106,128].

Paxillin. Paxillin plays a role in regulating integrin function in some haematopoietic cells. It binds downstream from the KxGFFKR regulatory domain to a hydrophobic Tyr residue and actively regulates cellular functions such as cell spreading or migration in inflammatory T-cells[129]. Phosphorylation can down-regulate paxillin binding and thereby modulate cellular responses to inflammation. Paxillin acts as a hub, binding both positive (FAK, Src and others) and negative (CSK, an inhibitor of Src, and various protein phosphatases) regulators of integrin signalling, bringing them into close proximity to their targets[130].

Strategies to therapeutically target integrins

There are three approaches to inhibiting integrin function. The original strategy, and the one used by all of the currently approved inhibitors, is blockade of ligand binding. However, with recent advances in our understanding of integrin signalling pathways the concept of blocking downstream integrin signalling has become attractive. A third approach is the modulation of integrin expression, which, although it has not been a specific strategy has been implicated in the effect of some drugs. Below we discuss these strategies, in particular with respect to the integrins for which inhibitors have been approved (α_4 integrins, $\alpha_L\beta_2$ and $\alpha_{IIb}\beta_3$).

Inhibition of ligand binding

Structural analysis of integrin antagonists. Both integrins and their ligands are large molecules, and thus there is the potential for competitive antagonists to interact with more than one site on the integrin in order to block ligand binding. Two key binding sites for the competitive antagonists are the α-I domain and β-I domain. Figure 4 illustrates the known binding sites for integrin antagonists. In α-I domain-containing integrins, competitive inhibitors compete for the ligand-binding MIDAS on the α-chain (FIG. 4 a-d). In integrins lacking the α-I domain, competitive antagonists occlude the MIDAS of the β-I domain on the β-chain (FIG. 4 e-h). Inhibitors that bind to sites distal to the MIDAS domains have been suggested to act by sterically blocking the MIDAS. Putative allosteric sites are indicated with red Xs in FIG. 4.

A major challenge associated with the development of integrin antagonists is the identification of activation-specific agents in addition to both subunit- and integrin complex-specific inhibitors. Below we have summarized the structural information associated primarily with approved drugs that target α_L, $\alpha_{IIb}\beta_3$ and α_4.

α-I domain-containing integrins: efalizumab. Efalizumab binds to the α_L subunit of $\alpha_L\beta_2$, which is a typical α-I domain containing integrin. The structure of efalizumab in complex with the α-I domain of the α_L subunit has been solved[131]. It binds distal to the MIDAS-domain; however, the crystalographic structure suggests that it acts to sterically hinder the binding of the $\alpha_L\beta_2$ ligand ICAM-1 (FIG. 5a). The crystal structure of efalizumab-bound α_L suggests that it is in the unliganded resting/closed conformation when compared to the resting[132] and activated

structures[133]. Efalizumab interacts with the α1 and α3 helices, and is thus thought to also act by stablizing the closed, low affinity conformation, thereby preventing changes in the ligand-binding region. Along with a number of hydrophobic interactions in the efalizumab binding site, the residues Asp193, Lys197, Lys200, His201 on α_L form hydrogen bonds and salt-bridges with efalizumab. These residues are not conserved in the α-I domains of other integrins, revealing the potential to design specific inhibitors by exploiting this region of α-I domains.

Inhibitors of the α/β-I domain linker site. There are also a group of small molecule α_L ligand mimetics based on the stucture of ICAM-1[134-135], which are proposed to bind competitively to the ICAM-1 binding site in the α-I domain[135]. It has also been suggested that these antagonists could bind to the ligand-binding site between the β-I like domain MIDAS and the β-propeller, also known as the α/β I-domain allosteric site or the *'linker site'* (see FIG. 4d)[136-139]. While inhibiting this *linker site* is an enticing prospect, no inhibitors of this region have been co-crystalized as yet.

Allosteric inhibitors. $\alpha_L\beta_2$ interacts with ICAM through its α-I domain. The carboxyl group of Glu34 on the D1 domain of ICAM-1 co-ordinates directly with the Mg^{2+} in the MIDAS domain of α_L[133]. Displacement of helices α1 and α7 occurs with ligand binding leading to receptor activation (see FIG. 3c). The α-I domain of the α_L subunit has been co-crystalized with a number of small molecule inhibitors[131,140-145] identifying the first model of true allosteric inhibition of an α-I domain (FIG. 5b). Crystallographic studies have demonstrated that the small molecules bind to a pocket in contact with the α7 helix on the opposite side of the molecule from the MIDAS

domain[140-145]. This region has been previously shown to be important for ligand binding and receptor function[146-147]. Binding of small molecules do not significantly change the MIDAS domain, suggesting that they do not activate the receptor. Lovastatin, a lipid-lowering drug that was found unexpectedly to inhibit binding of ICAM to $\alpha_{L,}$ and the volatile anesthetic isoflurane have both been co-crystallized in this position[142,148] and both have been shown to inhibit immune functioning[149-150]. Kallen *et al.* suggest that lovastatin may play a role in signalling by locking the molecule in an inactive, low-affinity form through stabilizing the C-terminal α7 helix[142].

Activation state-specific antagonists. It is possible to develop inhibitors that are selective for the activation state of the receptor[151]. Drugs that bind to the resting receptor can be effective even if there is a low plasma concentration of free drug although this may be associated with more adverse effects. Conversely, drugs that only bind to the activated receptor may have reduced adverse events but require a stable plasma concentration. The antibody AL-57 has been shown to bind specifically to the activated/open α_L MIDAS[152]. This inhibitor demonstrates the possibilities for developing competitive activation-specific inhibitors for the α-I domain.

β-I domain-containing integrins.

Integrins lacking the α-I domain bind their ligands between the β-propeller of the α subunit and the β-I domain (see FIG. 3b and 4e-h). Examples of therapeutically important β-I domain-containing integrins are the α_4 and β_3-containing integrins. In the case of β_3 integrins, the ligand interacts with the MIDAS of the β-I domain and an

acidic residue/residues on the β-propeller domain at the RGD ligand binding site (FIG. 5c). Tirofiban (see Table 1), eptifibatide and L-739758 have been crystalized in this position in $\alpha_{IIb}\beta_3$. The $\alpha_v\beta_3$ antagonist RGD-f-(NMe)V (cilengitide) has also been crystalized in a similar position (FIG. 5f). Eptifibatide competes directly for the ligand binding site inhibiting both $\alpha_{IIb}\beta_3$ and $\alpha_v\beta_3$, whereas tirofiban is specific for $\alpha_{IIb}\beta_3$ providing selectivity between the two receptors (see FIG. 5c & e). Abciximab binds to an alternative site on the β_3 subunit of both $\alpha_{IIb}\beta_3$ and $\alpha_v\beta_3$. This site is distal to the RGD-ligand binding site on the β_3 chain (FIG. 5d; 7E3 site). Its binding is thought to lead to steric hindrence of the fibrinogen macromolecule due to the proximity of residues involved to the RGD site[97,153].

The extracellular domain of $\alpha_v\beta_3$ receptor has been crystalized in the resting state[98], while the ligand-binding domain of $\alpha_{IIb}\beta_3$ has been crystalized in the activated form, and in complex with the ligand-mimetics tirofiban and eptifibatide[97]. These ligands bound to the same site co-ordinating with the Mg^{2+} ion associated with Ser123 through their carboxy groups, and with Asp224 on the β-propeller through their basic guanidinium group (figure 5E). This binding mode is analagous to cilengitide binding to $\alpha_v\beta_3$[154] (figure 5F). The guanidinum group of the arginine of cilengitide forms a bidentate salt bridge with Asp150 and Asp218. The carboxy group of the aspartic acid of cilengitide hydrogen bonds with the Mn^{2+} in the MIDAS, and the backbone amides of Tyr122 and Asn215[154]. This binding mode is very similar to that of the binding interaction for the RGD analogues in $\alpha_{IIb}\beta_3$.

α_4 antagonists. In addition to natalizumab, the approved mAb antagonist of α_4, small-molecule antagonists have been developed (see table 1). There is no crystal structure

data for any of the α_4 antagonists in complex with the integrin; however, a model has been proposed in which these small molecules bind to the Mg^{2+} of the MIDAS through their common carboxylic acid group analagous to the mechanism of many other integrin antagonists[155]. Removal of this carboxylic acid group decreased potency, but it also increased absorption, which has been a major stumbling block for the α_4 small molecule antagonists[156].

Selective antagonists. Even though many integrins share a subunit, such as $\alpha_{IIb}\beta_3$ and $\alpha_v\beta_3$, or can bind to the same motif, such as RGD, selective antagonists have been developed for many integrins. As $\alpha_v\beta_3$ is known to be important in angiogenesis, whereas $\alpha_{IIb}\beta_3$ is important in thrombosis, the development of selective integrin antagonists is vital. For example, unwanted inhibition of $\alpha_{IIb}\beta_3$ when targeting $\alpha_v\beta_3$ could lead to bleeding side effects. The easiest approach to developing a selective antagonist is with complex-specific monoclonal antibodies. A complex-specific $\alpha_4\beta_7$ antibody MLN-02 has shown efficacy in a Phase II trial for ulcerative colitis[157] and specific small-molecule antagonists for $\alpha_4\beta_7$ have been discovered[156].

While $\alpha_{IIb}\beta_3$ and $\alpha_v\beta_3$ share a subunit and both recognise the RGD-motif, specific antagonists of each receptor have been discovered. The selectivity of these antagonists was explored using a modelling approach[158] suggesting that selectivity is due to two main factors: first, the length between the acidic carboxyl group and the guanidinium group of the ligands that bind to the Asp148 and Arg214 integrin residues; and second, a $\pi-\pi$ stacking interaction between Tyr178 on the β-propeller and the ligand. There are no crystal structures available for the compounds the authors used in this study, or any other similar compounds binding in similar postions. It seems that

compounds obey the rule of binding through their basic group to an acid group on the β-propellor, and through their carboxyl group to the MIDAS in the β-I domain.

Analysis of the crystal structures of the ligand binding domain of $\alpha_{IIb}\beta_3$ and $\alpha_v\beta_3$ reveals significant differences in the distance between the acidic residues that interact with the ligand and the MIDAS domain. It appears that in $\alpha_v\beta_3$ 12.8-14.5 angstroms are required from the carbonyl of the carboxy group on the ligand to the nitrogen which hydrogen bonds with Asp148 or Arg214; while in $\alpha_{IIb}\beta_3$, a distance of 15.5-16.5 angstroms is required (FIG. S1; supplementary material). The difference between the average distances is 2.3 angstroms which equates to approximately 3 atoms on a carbon chain. This supports a study showing an $\alpha_{IIb}\beta_3$-specific antagonist with a linker of 15 atoms between the acidic and basic residues, while the $\alpha_v\beta_3$ specific antagonists generally only have a linker of 12 atoms[158].

There are other differences that affect selectivity such as hydrophobicity and the space available within the pockets of $\alpha_{IIb}\beta_3$ and $\alpha_V\beta_3$ integrins (FIG. S2; supplementary material). The amino acid Tyr178 in $\alpha_v\beta_3$ causes a bulge in the pocket which prevents large and inflexible subgroups from interacting with the acidic residues (Asp150 and Asp218). On the other hand, there appears to be more space in the 'chimney' region (Figure 5F). Therefore, selectivity seems to be achieved due to the properties of the binding pocket of the integrin, and the length of the linker between the acidic and basic groups on the ligand.

Receptor antagonists that do not induce conformational changes. A major problem with integrin antagonists is their potential to activate the receptor. All the clinically

approved β_3 antagonists act as partial agonists, activating signalling through allosteric changes in the β-chain[159]. Receptor activation can be measured using antibodies to epitopes exposed with receptor activation (ligand-induced binding sites; LIBS). For receptors containing a β-I domain such as $\alpha_{IIb}\beta_3$, it has been proposed that targeting the α-chain will prevent allosteric activation through the β-chain[160]. The antibody 10E5 binds to the β-propellor on the α-chain (see FIG. 5d). It acts by sterically blocking fibrinogen binding. This antibody is selective for α_{IIb} and, unlike abciximab, it does not stimulate LIBS expression.

A recently described $\alpha_{IIb}\beta_3$ small molecule antagonist was found not to induce LIBS exposure on the β_3 subunit[160]. Molecular docking studies suggested that this molecule was unlikely to interact with the MIDAS domain which is consistent with the lack of carboxyl group required for the interaction with the metal ion in the β-I domain (FIG. 5g). It is also too small to simultaneously bind to both the α-chain (β-propeller) and β-chain (β-I domain MIDAS). This is potentially the start of a paradigm shift for $\alpha_{IIb}\beta_3$ antagonists in which inhibitors could be designed to block ligand binding without interacting with the β-I domain on the β-chain of the integrin.

There is, however, another antagonist that does not induce α_{IIb} or β_3-LIBS expression despite it containing a carboxyl group and being large enough to stretch between Asp224 of the β-propellor and the MIDAS domain[161-162]. It is not clear why this small molecule (UR-2922) would not induce LIBS while others do, as it obeys the basic RGD-mimetic structure with an acidic group on one side, and a basic group on the other. Molecular docking studies in our laboratory predict that UR-2922 will interact

with the Asp224 in the β-propeller (FIG. 5h). The model predicts that UR-2922 does not interact with the MIDAS but rather, its carboxy group forms a salt bridge with Arg165 and a hydrogen bond with Tyr166. This supports the assertion by Blue *et al.* that preventing the interaction with the MIDAS can inhibit β_3 LIBS activation[160]. UR-2922 is also predicted to have an additional π–π stacking interaction with Phe160, where neither tirofiban, nor L-739758 do. This may explain the very strong binding affinity of UR-2922 (<1nM) and its slow dissociation rate (k_{off} = 90 min). Therefore, binding affinity may have more of a role than previously thought in LIBS activation and signalling.

Inhibition of integrin signalling and expression.

Although many current therapeutic strategies are aimed at blocking integrin association with ligands, there is a recent appreciation that an alternative strategy may be to target intracellular signalling mechanisms to inhibit integrin activation, thereby suppressing ligand binding, or to prevent the downstream sequela of integrin activation[116,163-165]. Potential strategies are focused on the short cytoplasmic tails of the integrin α and β subunits, although there is also evidence that the transmembrane domains may play a role[103-104,166].

As discussed earlier, talin binding is a critical event in integrin function and therefore represents a potential novel target for developing anti-integrin strategies. Similarly, kindlin or c-src binding to β-integrin tails and paxillin binding to α-integrin cytoplasmic tails represent additional targets for development of selective inhibitors

of integrin-signalling pathways. In particular, talin and paxillin represent hub proteins that facilitate the assembly of critical integrin downstream signal regulators. Inhibitors of interactions with these key proteins will prevent the coordinated assembly of focal adhesions critical for integrin function.

Other novel strategies are currently being explored to affect selective integrin inhibition. These include peptide inhibitors that mimic integrin cytoplasmic tails[106] and transmembrane helix modulators[95]. To date, the peptidomimetic compounds that have emerged from such programmes are not potent or specific enough to encourage further development. In contrast, inhibition of paxillin binding has been shown to selectively inhibit α_4-integrin-mediated responses in T-cells whilst maintaining α_4-independent cellular responses[167]. Recently, as proof-of-principle of the pharmacological use of such agents a selective small-molecule inhibitor of the paxillin interaction with the integrin α_4 cytoplasmic tail (see Table 1) has been shown to potently reduce T-cell migration *in vivo*[168].

The D-amino acid peptide KIKMVISWKG (HYD1) binds to $\alpha_6\beta_1$ and inhibits signaling but not ligand binding[169]. ATN-161 (Ac-PHSCN-NH_2) is a mimetic of the fibronectin sequence PHSRN undergoing clinical development for the treatment of solid tumours[170]. It appears to interact with β sub-units, blocking signalling rather than adhesion, and it is thought that this may be due to inhibition of the re-arrangement of disulphide bonds necessary for signalling in a similar manner to inhibitors of thiol isomerase action on integrins[164,171].

Integrin-linked kinase (ILK) is also a potential target in cancer[172-173], although there is the possibility of non-specific actions on other kinases. A number of small-molecule inhibitors of ILK have been tested in *in vitro* models of cancer and metastasis[174-175]. Compounds such as QLT0267, a pharmacological inhibitor of the kinase activity of ILK, abolishes downstream integrin signalling events such as phosphorylation of Akt/PKB and are proposed to have a strong potential role in treatment of acute myeloid leukemia[176].

Finally, modulation of integrin expression also has the potential to be an effective strategy. One such modulator is E7820 (see Table 1), which inhibits angiogenesis by reducing the expression of β_2 integrins[177].

Conclusions and future directions

The diversity of integrins and their complex role in many diseases suggests great potential for this superfamily as drug targets. However, while initially a promising strategy, successful therapeutic inhibition of integrins has proven to be elusive, despite the discovery of highly potent inhibitors. This is due to a number of reasons including redundancy among the integrins, the importance of integrins in key physiological systems and antagonists whose properties are less than optimal.

Redundancy among integrins may be impossible to overcome but the use of less selective inhibitors, such as the use of an anti-α_4 inhibitor rather than an $\alpha_4\beta_1$ inhibitor may prove to be more effective in some cases. However, care must be taken to monitor potential unwanted effects. Moreover, existing studies suggest that some integrins can play critical roles in many physiological systems and any inhibition of

these may result in serious adverse effects; for example the role of α_4 and $\alpha_L\beta_2$ integrins in normal B-cell function.

For antibody-based integrin-targeted drugs, there is a risk of the generation of neutralizing antibodies to the drug leading to reduced efficacy. This has been shown to happen with abciximab[178] and natalizumab[179]. In the case of abciximab, this can also lead to severe thrombocytopenia, although this is not solely due to anti-abciximab antibodies as it also occurs with small molecule $\alpha_{IIb}\beta_3$ antagonists[178].

For small-molecule integrin-targeted drugs, as many have been designed around the natural ligand for the receptors, such as RGD, it is not surprising that the resulting antagonists often display agonist-like activity[180]. RGD-based $\alpha_V\beta_3$ and $\alpha_V\beta_5$ inhibitors were found to stimulate angiogenesis at low doses[181]. This has also been seen with oral $\alpha_{IIb}\beta_3$ antagonists, for which low doses were shown to induce platelet aggregation while higher doses were inhibitory[182]. In both cases, the problems appear to arise during trough periods. In the case of $\alpha_{IIb}\beta_3$ antagonists, this is not a problem with the intravenous agents, as these are maintained at high plasma concentrations using an infusion. However, it was a major problem for the development of oral compounds[31].

It is noteworthy that the discovery and development of the first generation of anti-integrins occurred at a critical juncture in the pharmaceutical industry, as it was undergoing a transition from a chemistry-led to a target-led discovery strategy. Indeed many of the problems with the anti-integrins developed so far could be linked to the strategy of target-led discovery. Often inhibitors of newly discovered receptors are

developed without a detailed understanding of the receptor, and thus, the lack of understanding of integrin signalling led to the failures of many candidates due to agonist-like properties of the antagonists. However, more recent studies of the competitive and non-competitive ligand-binding sites should allow us to design more effective antagonists. Also, as discussed the growing body of knowledge of the integrin signalling pathways make it possible to consider targeting specific aspects of integrin signalling as an alternative to the traditional approach of receptor blockade.

A serious concern, however, is whether the pharmaceutical industry will take on board the lessons of the failed compounds and develop better antagonists. After the initial failure of the oral $\alpha_{IIb}\beta_3$ antagonists, xemilofiban and orbofiban, other companies continued to perform clinical studies on this class of drug, but appeared not to take account of the previous problems encountered or their causes. As a result the subsequent trials failed with increased mortality since the new compounds had the same problems[31]. However, when new-generation compounds such as UR-2922 were developed that had the potential to addressed all of the short comings of the earlier compounds[161], there was no appetite to develop them clinically.

In addition, although the success of natalizumab and efalizumab has shown the clinical relevance of the α_4 and $\alpha_L\beta_2$ integrins, and encouraged the search for small-molecule antagonists[183-185], the side-effects with both natalizumab and efalizumab could discourage companies from pursuing small-molecule antagonists for these targets even if they appear to have improved characteristics. Two key questions need to be addressed. Will small molecule inhibitors have the same propensity to cause PML? Is it possible to expand the therapeutic applications of such inhibitors? Given

the severity of diseases such as multiple sclerosis and the limited treatment options, identifying and addressing the problems with existing integrin-targeted therapies could be the key to developing successful treatments for these diseases.

Overall, the complexity and diversity of integrins provide great opportunities for drug development, but also major challenges. Nevertheless, a greater understanding of their activation mechanisms and structural information has opened new doors for the development of both allosteric and activation-specific drugs. This, coupled with possibilities of modulating integrin signalling, means that the potential of integrins as drug targets is far from exhausted.

Figure legends

Figure 1 | Integrin families. Integrins are heterodimers comprised of an α and β subunit, and at least twenty-four such heterodimers have been identified, which are shown here. Initially, integrin families were defined by their β subunit[2], but later it became apparent that the eighteen α subunits and ten β subunits formed specific but diverse heterodimers. Thus, α_V can associate with many different β subunits, whereas other α subunits are singular in their choice of β partner. Moreover, the α subunit appears to be important in defining the ligand binding properties of the integrin. Consequently, defining integrin families requires a more flexible system taking into consideration their ligand-binding properties and functions.

Figure 2 | Integrin structure and changes upon activation. This figure is based on the structures of $\alpha_V\beta_3$ and $\alpha_{IIb}\beta_3$, adapted from Xiao *et al*.[97] and Shimaoka *et al*.[102] The overall integrin sequence structure is outlined in **a**, and the three main activation states are shown in **b**. The *'resting'* state is represented by the bent, closed conformation, which has a low affinity for the ligand. The *'intermediate'* state is extended; however, the headpiece is still 'closed', and therefore this state also has a low affinity for the ligand. In the fully *'activated'* state, the hybrid domain of the thigh domain swings out by 62° and the α 7 helix moves downwards, opening up the head domain into a high affinity state. **c** | A model of the activation due to 'outside-in' signalling (top), and 'inside-out' signalling (bottom), based on the proposal by Xiao *et*

al[97]. The model represents a number of intermediates between the three main activation states outlined in **b**.

Figure 3 | Structure and function of integrin α-I and β-I domains. Structures are displayed as cartoon ribbons or as surface representations. Helices are represented in pink, β-sheets in grey, and turns in cyan. The metal ion of the MIDAS is displayed in blue and the calcium ions are displayed in cyan. **a |** Representative structure of an α-I domain (α_L 1CPQ). **b |** Representative structure of the head domain an integrin lacking an α-I domain. This integrin contains an I-like domain on the β-chain known as the β-I domain. The integrin used in this example is the $\alpha_{IIb}\beta_3$ head domain (2VDK). The β-propeller surface is displayed as a blue surface complexed with the β-I domain displayed as a ribbon structure. The ligand binding site stretches between the β-propeller domain and the β-I domain contacting the Mg^{2+} ion of the MIDAS. **c |** Cartoon displaying the major changes that occur to the α1 and α7 helices with receptor activation. This image was generated from an overlay of the open (1IDO) and closed (1JLM) structure of α_M.

Figure 4 | Integrin activation states and inhibitor sites. Panels **a** to **d** represent integrins containing the inserted α-I domain, based on the model proposed for the complement receptor 4[186]. Panels **e** to **h** represent integrins lacking the α-I domain based on the structures of $\alpha_V\beta_3$ and $\alpha_{IIb}\beta_3$, and the model proposed by Xiao *et al.*[97] Ligand binding regions are indicated by the grey oval areas. Integrins containing the α-I domain are thought to have a great deal of freedom of their α-I domain in the closed state indicated by the arrow (**a**). Activation of the β-I domain MIDAS site and

interaction between the MIDAS and the acidic residue (at the *'linker site'*) locks the α-I domain at an angle with high strain (**b**). Movement of the α7 helix downwards due to the activation of the α-I MIDAS site leads to release of this tension, and a reduction in the angle of the α-I domain to the β-I domain (**c**). The model for the integrins lacking the inserted α-I domain is simpler with activation of the β-I MIDAS leading to increased affinity for ligands (**f**). The α7 helix also moves down with activation of the MIDAS leading to allosteric changes in the molecule resulting in activating signalling events within the cell (**g**). Inhibition sites for each group of integrins that have been identified are indicated with an 'X' (**d** and **h**). Putative sites that have been proposed are identified with a red 'X'.

Figure 5 | Structural analysis of inhibitors of integrins. Panels **a**,**b** represent α-I domain inhibitor sites on α_L, while **c**-**f** represent β-I domain inhibitor binding sites on $\alpha_{IIb}\beta_3$ and $\alpha_v\beta_3$. Structures are displayed as cartoon ribbons or as surface representations. Helices are represented in pink, β-sheets in grey, and turns in cyan. The metal ion of the MIDAS is displayed as blue and the calcium ions are displayed as cyan. When ligands are displayed, carbons are coloured green or grey, oxygens red, nitrogens blue, and hydrogens white. **a |** Binding site of the antibody efalizumab to the α-I domain of α_L. The structure of α_L (3EOB) has been displayed with the area identified to interact with efalizumab represented as a surface and coloured according to amino acid residue type; acidic residues-red,basic residues-blue, polar residues-white, and non-polar residues green. **b |** Allosteric inhibition of the α-I domain of α_L by small molecules. Lovastatin is shown bound to the α-I domain of α_L (1CQP) as a representative molecule of allosteric inhibitors that bind to this site. **c |** Structure of

the head domain of $\alpha_{IIb}\beta_3$ in complex with tirofiban (2VDM). D224 is rendered in red. **d |** Summary image depicting the orientation of binding sites for the antibodies 10E5 and 7E3 (abciximab site). The interacting region is displayed as a surface coloured according to amino acid residue type as in **a**. The representative small molecule competitive antagonist, tirofiban is displayed to highlight the ligand binding pocket. **e |** Surface representation of the binding site for tirofiban in $\alpha_{IIb}\beta_3$. Positively charged regions are coloured red, negatively charged areas blue, and neutral regions white. **f |** cycloRGD-f-(N-Me)V bound to $\alpha_V\beta_3$. **g |** Docking pose of compound 1 in the $\alpha_{IIb}\beta_3$ binding pocket[160]. **h |** Predicted binding position of UR-2922. These images were generated using Visual Molecular Dynamics software[187] and Molecular Operating Environment, CCG, Montreal.

Box 1 | Choreography of $\alpha_{IIb}\beta_3$-mediated signalling

As shown in panel **a** of the figure, the default conformation of the platelet-specific $\alpha_{IIb}\beta_3$ integrin in unactivated platelets is maintained by clasps in the cytoplasmic tails[94], whereby the integrin α and β cytoplasmic tails form a salt bridge between the highly conserved β-HDRKE motif and the arginine in the α- KVGFFKR motif[188]. Following separation of the two integrin tails by activation events that direct talin binding to the C-terminal NPXY phosphotyrosine domain of the β-integrin tail, the membrane proximal regions of both α and β become available to bind a range of proteins[105,109], including tensin, filamin, paxillin and, either directly or indirectly, focal-adhesion kinase (FAK). Talin then binds a second β-integrin binding site closer to the membrane and initiates a second phase of activation/regulatory events. Phosphorylation of the proximal β-NPXY motifs acts as a regulatory switch for adaptor binding[110,189]. Kindlins bind to the distal NXXY motif and are co-activators

with talin and tensin[119]. A unique role for c-src has been demonstrated for $\alpha_{IIb}\beta_3$. C-src binds to the C-terminal tip of the β-CT and regulates the capacity of this integrin subunit to be phosphorylated.

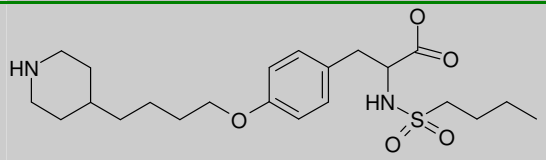
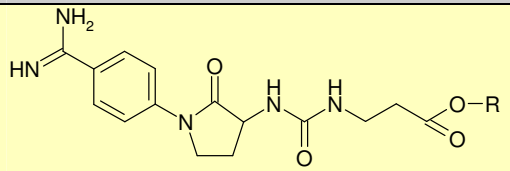
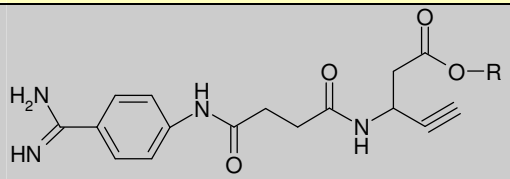
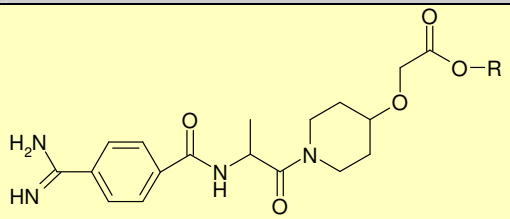
To date, there are 7 platelet proteins known to interact with the regulatory KVGFFKR domain of α_{IIb} integrin cytoplasmic tail. These include CIB1[190], AUP-1[191], PP1C[192], PP2A[193], TIM[194], ICln[195] and RN181[196]. In addition, paxillin binds more distally and regulates the capacity of the integrin to transduce strain via talin associations with the cytoskeleton[130,167]. Paxillin acts as a hub, binding both positive (FAK, Src and others) and negative (CSK, an inhibitor of Src, and various protein phosphatases) regulators of integrin signalling, bringing them into close proximity to their targets. Thus, the α-integrin tail is a molecular scaffold for the coordination of interactions of cytoplasmic regulatory proteins to promote or modulate integrin responses. A controlled choreography of binding is apparent, showing that CIB1 and protein phosphatases bind to the αβ-CT complex in resting integrins, while ICln only binds transiently following activation[195]. Targeting any of these specific interactions has the potential to disrupt integrin activation therapeutically. As shown in panels **b** and **c**, the cytoplasmic membrane-adjacent regions are highly conserved in integrin α and β subunits and act as a docking point for many cytoplasmic proteins.

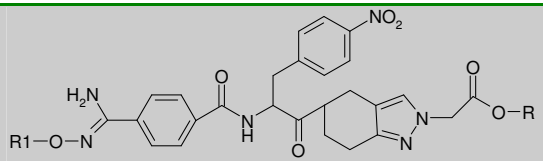
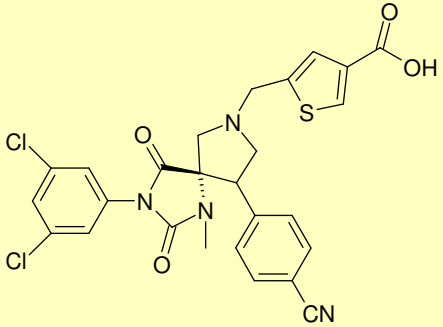
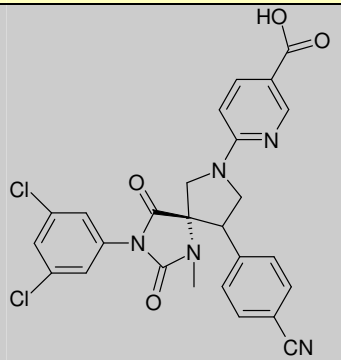
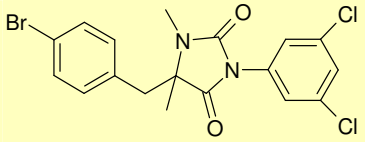
Figure S1: Analysis of the lengths of β_3 antagonists. a-c represent ligands bound to $\alpha_{IIb}\beta_3$ while **d** represents cycloRGD-f-(NMe)V bound to $\alpha_V\beta_3$. The ligands are coloured according to atom type with oxygen coloured red, nitrogen blue and sulphur yellow. The carbon atoms are coloured orange in tirofiban **a**, green in eptifibatide **b**,

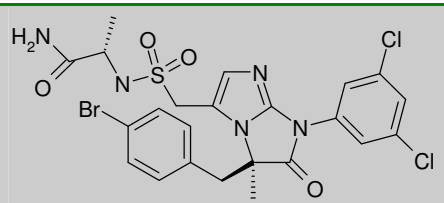
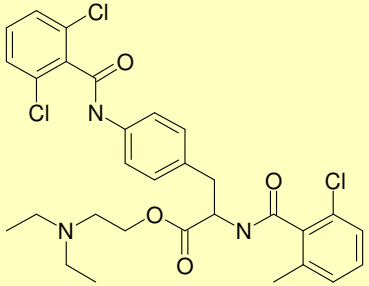
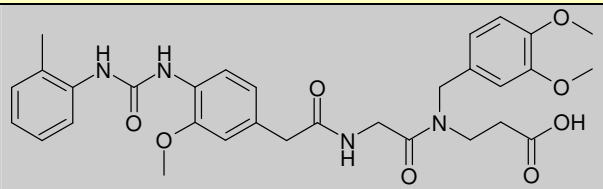
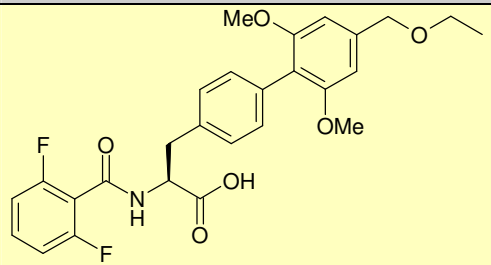
grey in L-739758 **c** and purple in cycloRGD-f-(NMe)V. Distances between the carbonyl of the carboxy group interacting with the MIDAS and the nitrogens involved in hydrogen bonding with the Asp218 or Asp150 on $\alpha_V\beta_3$ or Asp224 on $\alpha_{IIb}\beta_3$ labeled in a-d are summarized in the table.

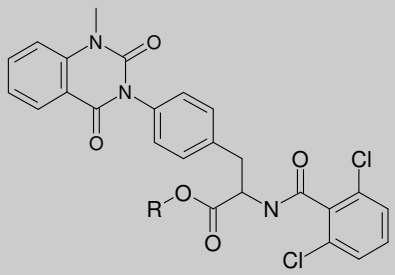
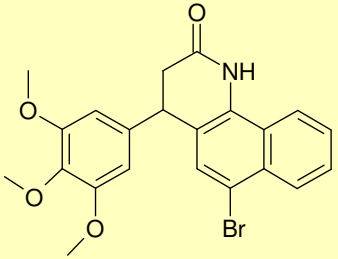
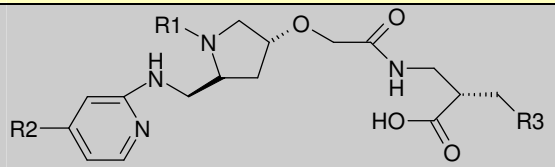
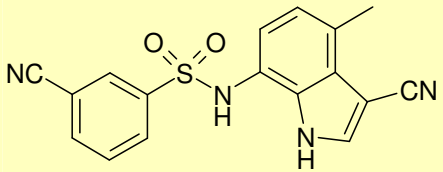
Figure S2: Structural overlay of the binding pocket of $\alpha_{IIb}\beta_3$ and $\alpha_V\beta_3$. a represents the crystal structure of the cyclo-RGD-f-(N-Me)V bound to $\alpha_V\beta_3$**. b** depicts the binding pocket of $\alpha_{IIb}\beta_3$ in complex with tirofiban. The surface of the $\alpha_V\beta_3$ pocket is coloured red, and the surface of $\alpha_{IIb}\beta_3$ is coloured cyan **b**. The two structures are overlaid in **C** to highlight the spacial differences in the pocket. The ligands are coloured according to atom type: oxygen- red, nitrogen- blue, sulphur-yellow. Carbon atoms are coloured purple in cyclo RGD-f-(N-Me)V, and orange in tirofiban. The metal ions are depicted in space fill with Ca^{2+} coloured cyan, Mg^{2+} coloured blue and Mn^{2+} coloured orange.

Table 1. Structure of small, non-peptide inhibitors of integrins that have undergone clinical development.

Name	Structure	Company	Stage	Indication	Target	References
Tirofiban		Merck	Approved	Thrombosis	$\alpha_{IIb}\beta_3$	[197]
Orbofiban		Searle	Dropped PIII	Thrombosis	$\alpha_{IIb}\beta_3$	[198]
Xemilofiban		Searle	Dropped PIII	Thrombosis	$\alpha_{IIb}\beta_3$	[199]
Sibrafiban		Roche	Dropped PIII	Thrombosis	$\alpha_{IIb}\beta_3$	[200]

UR-3216	 R= ethyl and R1 = OH	Ube	Dropped Pre-clinical	Thrombosis	$\alpha_{IIb}\beta_3$	**161**
BMS-587101		Bristol-Myer Squibb	Dropped PII	Psoriasis	$\alpha_L\beta_2$	**143**
BMS-688521		Bristol-Myer Squibb	Pre-clinical	Psoriasis	$\alpha_L\beta_2$	**185**
BIRT 377		Boehringer Ingelheim	Dropped	Psoriasis	$\alpha_L\beta_2$	**201**

BIRT 2584		Boehringer Ingelheim	PI Dropped	Psoriasis	$\alpha_L\beta_2$	202
R411; Valategrast		Roche	Dropped PII	Asthma	$\alpha_4\beta_1$	184
IVL745		Sanofi-Aventis	Dropped PII	Asthma	$\alpha_4\beta_1$	184
Firategrast (SB-683699)		GSK	PII	Multiple sclerosis	$\alpha_4\beta_1$	184

AJM300		Ajinomoto	PII	Ulcerative colitis	$\alpha_4\beta_1$	184
6-B345TTQ			Pre-clinical		α_4–paxillin interaction	168
JSM6427		Jerini AG	PI	Age-related macular Degeneration	$\alpha_5\beta_1$	203
E7820		Eisai	PI	Solid tumours	α_2 (inhibition of expression)	177

References

1. Tamkun, J.*, et al.* Structure of integrin, a glycoprotein involved in the transmembrane linkage between fibronectin and actin. *Cell* **46**, 271-282. (1986).
2. Ruoslahti, E. & Pierschbacher, M.D. New perspectives in cell adhesion: RGD and integrins. *Science* **238**, 491-497 (1987).
3. Bonaca, M.P.*, et al.* Antithrombotics in Acute Coronary Syndromes. *J Am Coll Cardiol* **54**, 969-984 (2009).
4. Chua, D. & Ignaszewski, A. Clopidogrel in acute coronary syndromes. *Br Med J* **338**, b1180 (2009).
5. Rice, G.P.A., Hartung, H.-P. & Calabresi, P.A. Anti-α4 integrin therapy for multiple sclerosis: Mechanisms and rationale. *Neurology* **64**, 1336-1342 (2005).
6. Targan, S.R.*, et al.* Natalizumab for the treatment of active Crohn's disease: results of the ENCORE Trial. *Gastroenterol* **132**, 1672-1683 (2007).
7. Frampton, J.E. & Plosker, G.L. Efalizumab: A Review of its Use in the Management of Chronic Moderate-to-Severe Plaque Psoriasis. *Am J Clin Dermatol* **10**, 51-72 (2009).
8. Warnke, C.*, et al.* Natalizumab and progressive multifocal leukoencephalopathy: what are the causal factors and can it be avoided? *Arch Neurol* **67**, 923-930 (2010).
9. Pytela, R., Pierschbacher, M.D. & Ruoslahti, E. A 125/115-kDa cell surface receptor specific for vitronectin interacts with the arginine-glycine-aspartic acid adhesion sequence derived from fibronectin. *Proc Nat Acad Sci U S A* **82**, 5766-5770 (1985).
10. White, D.J., Puranen, S., Johnson, M.S. & Heino, J. The collagen receptor subfamily of the integrins. *The International Journal of Biochemistry & Cell Biology* **36**, 1405-1410 (2004).
11. Evans, R.*, et al.* Integrins in immunity. *J Cell Sci* **122**, 215-225 (2009).
12. Brennan, M., Moriarty, R., Grennan, S., Chubb, A. & Cox, D. C-reactive protein binds to $\alpha_{IIb}\beta_3$. *J Thromb Haemost* **6**, 1239-1241 (2008).
13. Coller, B.S. & Shattil, S.J. The GPIIb/IIIa (integrin $\alpha_{IIb}\beta_3$) odyssey: a technology-driven saga of a receptor with twists, turns, and even a bend. *Blood* **112**, 3011-3025 (2008).
14. Takagi, J. Structural basis for ligand recognition by RGD (Arg-Gly-Asp)-dependent integrins. *Biochem. Soc. Trans.* **32**, 403-406 (2004).
15. Hynes, R.O. Integrins: Bidirectional, Allosteric Signaling Machines. *Cell* **110**, 673-687 (2002).
16. Spencer, T.E., Johnson, G.A., Bazer, F.W. & Burghardt, R.C. Implantation mechanisms: insights from the sheep. *Reproduction* **128**, 657-668 (2004).
17. Silva, R., D'Amico, G., Hodivala-Dilke, K.M. & Reynolds, L.E. Integrins: The Keys to Unlocking Angiogenesis. *Arterioscler Thromb Vasc Biol* **28**, 1703-1713 (2008).

18. Smith-Garvin, J.E., Koretzky, G.A. & Jordan, M.S. T Cell Activation. *Ann Rev Immunol* **27**, 591 (2009).
19. Furie, B. Pathogenesis of thrombosis. *Hematology* **2009**, 255-258 (2009).
20. Phillips, D.R. & Agin, P.P. Platelet Membrane Defects in Glanzmann's Thrombasthenia :Evidence for decreased amounts of two major glycoproteins. *J Clin Invest* **60**, 535-545 (1977).
21. Curtin, R. Intravenous glycoprotein IIb/IIIa antagonists: their benefits, problems and future developments. *Curr Pharm Des* **10**, 1577-1585 (2004).
22. Use of a monoclonal antibody directed against the platelet glycoprotein IIb/IIIa receptor in high-risk coronary angioplasty. The EPIC Investigation. *N Engl J Med* **330**, 956-961 (1994).
23. Umans, V.A., Kloeg, P.H. & Bronzwaer, J. The CAPTURE trial. *Lancet* **350**, 445 (1997).
24. Scarborough, R.M. Development of eptifibatide. *Am Heart J* **138**, 1093-1104 (1999).
25. Randomised placebo-controlled trial of effect of eptifibatide on complications of percutaneous coronary intervention: IMPACT-II. Integrilin to Minimise Platelet Aggregation and Coronary Thrombosis-II. *Lancet* **349**, 1422-1428 (1997).
26. O'Shea, J.C.*, et al.* Platelet glycoprotein IIb/IIIa integrin blockade with eptifibatide in coronary stent intervention: the ESPRIT trial: a randomized controlled trial. *J Am Med Assoc* **285**, 2468-2473 (2001).
27. Inhibition of platelet glycoprotein IIb/IIIa with eptifibatide in patients with acute coronary syndromes. The PURSUIT Trial Investigators. Platelet Glycoprotein IIb/IIIa in Unstable Angina: Receptor Suppression Using Integrilin Therapy. *N Engl J Med* **339**, 436-443 (1998).
28. Effects of platelet glycoprotein IIb/IIIa blockade with tirofiban on adverse cardiac events in patients with unstable angina or acute myocardial infarction undergoing coronary angioplasty. The RESTORE Investigators. Randomized Efficacy Study of Tirofiban for Outcomes and REstenosis. *Circ* **96**, 1445-1453 (1997).
29. Inhibition of the platelet glycoprotein IIb/IIIa receptor with tirofiban in unstable angina and non-Q-wave myocardial infarction. Platelet Receptor Inhibition in Ischemic Syndrome Management in Patients Limited by Unstable Signs and Symptoms (PRISM-PLUS) Study Investigators. *N Engl J Med* **338**, 1488-1497 (1998).
30. Chew, D.P., Bhatt, D.L., Sapp, S. & Topol, E.J. Increased mortality with oral platelet glycoprotein IIb/IIIa antagonists: a meta-analysis of phase III multicenter randomized trials. *Circ* **103**, 201-206 (2001).
31. Cox, D. Oral GPIIb/IIIa antagonists: what went wrong? *Curr Pharm Des* **10**, 1587-1596 (2004).
32. Etzioni, A. Defects in the leukocyte adhesion cascade. *Clin Rev Allergy Immunol* **38**, 54-60 (2010).
33. Kent, S.J.*, et al.* A monoclonal antibody to α4 integrin suppresses and reverses active experimental allergic encephalomyelitis. *J Neuroimmunol* **58**, 1-10 (1995).
34. Bennett, J. Natalizumab and progressive multifocal leukoencephalopathy: migrating towards safe adhesion molecule therapy in multiple sclerosis. *Neurol Res* **28**, 291-298 (2006).

35. Gonzalez-Amaro, R., Mittelbrunn, M. & Sanchez-Madrid, F. Therapeutic anti-integrin (α4 and αL) monoclonal antibodies: two-edged swords? *Immunology* **116**, 289-296 (2005).
36. Steinman, L. Blocking adhesion molecules as therapy for multiple sclerosis: natalizumab. *Nat Rev Drug Discovery* **4**, 510 (2005).
37. Havrdova, E.*, et al.* Effect of natalizumab on clinical and radiological disease activity in multiple sclerosis: a retrospective analysis of the Natalizumab Safety and Efficacy in Relapsing-Remitting Multiple Sclerosis (AFFIRM) study. *Lancet Neurol* **8**, 254-260 (2009).
38. Polman, C.H.*, et al.* A randomized, placebo-controlled trial of natalizumab for relapsing multiple sclerosis. *N Engl J Med* **354**, 899-910 (2006).
39. Rudick, R.A.*, et al.* Natalizumab plus interferon β1a for relapsing multiple sclerosis. *N Engl J Med* **354**, 911-923 (2006).
40. Barthel, S.R., Johansson, M.W., McNamee, D.M. & Mosher, D.F. Roles of integrin activation in eosinophil function and the eosinophilic inflammation of asthma. *J Leukoc Biol* **83**, 1-12 (2008).
41. Tan, C.S. & Koralnik, I.J. Progressive multifocal leukoencephalopathy and other disorders caused by JC virus: clinical features and pathogenesis. *The Lancet Neurology* **9**, 425-437 (2010).
42. Multiple sclerosis woes. *Nat Neurosci* **8**, 837-837 (2005).
43. Yao, K.*, et al.* Reactivation of Human Herpesvirus-6 in Natalizumab Treated Multiple Sclerosis Patients. *PLoS ONE* **3**, e2028 (2008).
44. Leonardi, C.L.*, et al.* Extended efalizumab therapy improves chronic plaque psoriasis: Results from a randomized phase III trial. *J Am Acad Dermatol* **52**, 425-433 (2005).
45. Dubertret, L.*, et al.* CLinical experience acquired with the efalizumab (Raptiva) (CLEAR) trial in patients with moderate-to-severe plaque psoriasis: results from a phase III international randomized, placebo-controlled trial. *Br J Dermatol* **155**, 170-181 (2006).
46. Lebwohl, M.*, et al.* A novel targeted T-cell modulator, efalizumab, for plaque psoriasis. *N Engl J Med* **349**, 2004-2013 (2003).
47. Gordon, K.B.*, et al.* Efalizumab for patients with moderate to severe plaque psoriasis: a randomized controlled trial. *J Am Med Assoc* **290**, 3073-3080 (2003).
48. Ortonne, J.P., Shear, N., Shumack, S. & Henninger, E. Impact of efalizumab on patient-reported outcomes in high-need psoriasis patients: results of the international, randomized, placebo-controlled Phase III Clinical Experience Acquired with Raptiva (CLEAR) trial [NCT00256139]. *BMC Dermatol* **5**, 13 (2005).
49. Leonardi, C.*, et al.* Efalizumab: results of a 3-year continuous dosing study for the long-term control of psoriasis. *Br J Dermatol* **158**, 1107-1116 (2008).
50. Major, E.O. Progressive Multifocal Leukoencephalopathy in Patients on Immunomodulatory Therapies*. *Annual Review of Medicine* **61**, 35-47 (2010).
51. Moschos, S., Drogowski, L., Reppert, S. & Kirkwood, J. Integrins and cancer. *Oncology (Williston Park)* **21**, 13-20 (2007).
52. Jin, H. & Varner, J. Integrins: roles in cancer development and as treatment targets. *Br J Cancer* **90**, 561-565 (2004).
53. Avraamides, C.J., Garmy-Susini, B. & Varner, J.A. Integrins in angiogenesis and lymphangiogenesis. *Nat Rev Cancer* **8**, 604-617 (2008).

54. Axelsson, R., Bach-Gansmo, T., Castell-Conesa, J. & McParland, B.J. An open-label, multicenter, phase 2a study to assess the feasibility of imaging metastases in late-stage cancer patients with the αvβ3-selective angiogenesis imaging agent 99mTc-NC100692. *Acta Radiologica* **51**, 40-46 (2010).
55. Kenny, L.M.*, et al.* Phase I Trial of the Positron-Emitting Arg-Gly-Asp (RGD) Peptide Radioligand 18F-AH111585 in Breast Cancer Patients. *J Nucl Med* **49**, 879-886 (2008).
56. Nemeth, J.A.*, et al.* α_V Integrins as Therapeutic Targets in Oncology. *Cancer Investigation* **25**, 632 - 646 (2007).
57. Chudakova, D.A.*, et al.* Integrin-associated Lyn Kinase Promotes Cell Survival by Suppressing Acid Sphingomyelinase Activity. *J. Biol. Chem.* **283**, 28806-28816 (2008).
58. Koistinen, P., Ahonen, M., Kähäri, V. & Heino, J. α_V integrin promotes *in vitro* and *in vivo* survival of cells in metastatic melanoma. *Int J Cancer* **112**, 61-70 (2004).
59. Moschos, S.J.*, et al.* Pharmacodynamic (Phase 0) Study Using Etaracizumab in Advanced Melanoma. *J Immunother* **33**, 316-325 (2010).
60. Hersey, P.*, 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* **116**, 1526-1534 (2010).
61. Delbaldo, C.*, et al.* Phase I and pharmacokinetic study of etaracizumab (Abegrin), a humanized monoclonal antibody against $\alpha_V\beta_3$ integrin receptor, in patients with advanced solid tumors. *Invest New Drugs* **26**, 35-43 (2008).
62. Stupp, R.*, et al.* Phase I/IIa Study of Cilengitide and Temozolomide With Concomitant Radiotherapy Followed by Cilengitide and Temozolomide Maintenance Therapy in Patients With Newly Diagnosed Glioblastoma. *J Clin Oncol* **28**, 2712-2718 (2010).
63. Reardon, D.A.*, et al.* Randomized Phase II Study of Cilengitide, an Integrin-Targeting Arginine-Glycine-Aspartic Acid Peptide, in Recurrent Glioblastoma Multiforme. *J Clin Oncol* **26**, 5610-5617 (2008).
64. MacDonald, T.J.*, et al.* Phase I Clinical Trial of Cilengitide in Children With Refractory Brain Tumors: Pediatric Brain Tumor Consortium Study PBTC-012. *J Clin Oncol* **26**, 919-924 (2008).
65. Carter, A. Integrins as Target: First Phase III Trial Launches, but Questions Remain. *J. Natl. Cancer Inst.* **102**, 675-677 (2010).
66. Rosenthal, M.*, et al.* Evaluation of the safety, pharmacokinetics and treatment effects of an alpha(nu)beta(3) integrin inhibitor on bone turnover and disease activity in men with hormone-refractory prostate cancer and bone metastases. *Asia Pac J Clin Oncol* **6**, 42-48 (2010).
67. Kim, S., Harris, M. & Varner, J.A. Regulation of Integrin $\alpha_V\beta_3$-mediated Endothelial Cell Migration and Angiogenesis by Integrin $\alpha_5\beta_1$ and Protein Kinase A. *J. Biol. Chem.* **275**, 33920-33928 (2000).
68. Kim, S., Bell, K., Mousa, S.A. & Varner, J.A. Regulation of Angiogenesis in Vivo by Ligation of Integrin $\alpha_5\beta_1$ with the Central Cell-Binding Domain of Fibronectin. *Am J Pathol* **156**, 1345-1362 (2000).
69. Ng, C., Bai, S., Takimoto, C., Tang, M. & Tolcher, A. Mechanism-based receptor-binding model to describe the pharmacokinetic and pharmacodynamic of an anti-α5β1 integrin monoclonal antibody (volociximab) in cancer patients. *Cancer Chemother Pharmacol* **65**, 207-217 (2009).

70. Ricart, A.D., *et al.* Volociximab, a Chimeric Monoclonal Antibody that Specifically Binds {alpha}5{beta}1 Integrin: A Phase I, Pharmacokinetic, and Biological Correlative Study. *Clin Cancer Res* **14**, 7924-7929 (2008).
71. Laurens, N., *et al.* Single and combined effects of αvβ3- and α5β1-integrins on capillary tube formation in a human fibrinous matrix. *Angiogenesis* (2009).
72. Stewart, P.L. & Nemerow, G.R. Cell integrins: commonly used receptors for diverse viral pathogens. *Trends Microbiol* **15**, 500-507 (2007).
73. Fitzgerald, J.R., Foster, T.J. & Cox, D. The interaction of bacterial pathogens with platelets. *Nat Rev Microbiol* **4**, 445-457 (2006).
74. Loughman, A., *et al.* Roles for fibrinogen, immunoglobulin and complement in platelet activation promoted by *Staphylococcus aureus* clumping factor A. *Mol Microbiol* **57**, 804-818 (2005).
75. Brennan, M.P., *et al.* Elucidating the role of *S. epidermidis* serine-aspartate repeat protein G (SdrG) in platelet activation. *J Thromb Haemost* **9999**(2009).
76. Fitzgerald, J.R., *et al.* Fibronectin-binding proteins of *Staphylococcus aureus* mediate activation of human platelets via fibrinogen and fibronectin bridges to integrin GPIIb/IIIa and IgG binding to the FcγRIIa receptor. *Mol Microbiol* **59**, 212-230 (2006).
77. Yoon, C.S., Kim, K.D., Park, S.N. & Cheong, S.W. α6 Integrin is the main receptor of human papillomavirus type 16 VLP. *Biochem Biophys Res Commun* **283**, 668-673 (2001).
78. Fleming, F.E., Graham, K.L., Taniguchi, K., Takada, Y. & Coulson, B.S. Rotavirus-neutralizing antibodies inhibit virus binding to integrins α2β1 and α4β1. *Archives of Virology* **152**, 1087 (2007).
79. Graham, K.L., *et al.* Rotaviruses interact with $\alpha_4\beta_7$ and $\alpha_4\beta_1$ integrins by binding the same integrin domains as natural ligands. *J Gen Virol* **86**, 3397-3408 (2005).
80. Graham, K.L., *et al.* Integrin-Using Rotaviruses Bind $\alpha_2\beta_1$ Integrin α_2 I Domain via VP4 DGE Sequence and Recognize $\alpha_X\beta_2$ and $\alpha_V\beta_3$ by Using VP7 during Cell Entry. *J. Virol.* **77**, 9969-9978 (2003).
81. Schornberg, K.L., *et al.* $\alpha_5\beta_1$-Integrin controls Ebolavirus entry by regulating endosomal cathepsins. *Proc Nat Acad Sci* **106**, 8003-8008 (2009).
82. Dileepan, T., Kachlany, S.C., Balashova, N.V., Patel, J. & Maheswaran, S.K. Human CD18 Is the Functional Receptor for *Aggregatibacter actinomycetemcomitans* Leukotoxin. *Infect. Immun.* **75**, 4851-4856 (2007).
83. Dassanayake, R.P., Maheswaran, S.K. & Srikumaran, S. Monomeric Expression of Bovine β_2-Integrin Subunits Reveals Their Role in *Mannheimia haemolytica* Leukotoxin-Induced Biological Effects. *Infect. Immun.* **75**, 5004-5010 (2007).
84. Morova, J., Osicka, R., Masin, J. & Sebo, P. RTX cytotoxins recognize β_2 integrin receptors through N-linked oligosaccharides. *Proc Nat Acad Sci* **105**, 5355-5360 (2008).
85. Sewald, X., *et al.* Integrin Subunit CD18 Is the T-Lymphocyte Receptor for the *Helicobacter pylori* Vacuolating Cytotoxin. *Cell Host & Microbe* **3**, 20-29 (2008).
86. Kwok, T., *et al.* Helicobacter exploits integrin for type IV secretion and kinase activation. *Nature* **449**, 862-866 (2007).

87. Xudong, L. & Yinduo, J. Involvement of $\alpha_5\beta_1$-integrin and TNF-α in *Staphylococcus aureus* α-toxin-induced death of epithelial cells. *Cellular Microbiology* **9**, 1809-1821 (2007).
88. Martchenko, M., Jeong, S.-Y. & Cohen, S.N. Heterodimeric integrin complexes containing β1-integrin promote internalization and lethality of anthrax toxin. *Proceedings of the National Academy of Sciences*.
89. Jia, W., Li, H. & He, Y.-W. The extracellular matrix protein mindin serves as an integrin ligand and is critical for inflammatory cell recruitment. *Blood* **106**, 3854-3859 (2005).
90. Li, Y.*, et al.* Structure of the F-spondin domain of mindin, an integrin ligand and pattern recognition molecule. *EMBO J* **28**, 286-297 (2009).
91. Lee, H.-J.*, et al.* Polymorphisms and haplotypes of integrin α_1 (ITGA1) are associated with bone mineral density and fracture risk in postmenopausal Koreans. *Bone* **41**, 979-986 (2007).
92. Tofteng, C.L.*, et al.* Integrin β_3 Leu33Pro polymorphism and risk of hip fracture: 25 years follow-up of 9233 adults from the general population. *Pharmacogenetics and Genomics* **17**, 85-91 (2007).
93. Murphy, M.G.*, et al.* Effect of L-000845704, an $\alpha_V\beta_3$ integrin antagonist, on markers of bone turnover and bone mineral density in postmenopausal osteoporotic women. *J Clin Endocrinol Metab* **90**, 2022-2028 (2005).
94. Shattil, S.J., Kim, C. & Ginsberg, M.H. The final steps of integrin activation: the end game. *Nat Rev Mol Cell Biol* **11**, 288-300 (2010).
95. Caputo, G.A.*, et al.* Computationally designed peptide inhibitors of protein-protein interactions in membranes. *Biochemistry* **47**, 8600-8606 (2008).
96. Takagi, J., Petre, B.M., Walz, T. & Springer, T.A. Global conformational rearrangements in integrin extracellular domains in outside-in and inside-out signaling. *Cell* **110**, 599-511 (2002).
97. Xiao, T., Takagi, J., Coller, B.S., Wang, J.H. & Springer, T.A. Structural basis for allostery in integrins and binding to fibrinogen-mimetic therapeutics. *Nature* **432**, 59-67 (2004).
98. Xiong, J.P.*, et al.* Crystal structure of the extracellular segment of integrin $\alpha_V\beta_3$. *Science* **294**, 339-345 (2001).
99. Wang, W. & Luo, B.H. Structural basis of integrin transmembrane activation. *J Cell Biochem* **109**, 447-452 (2010).
100. Rosano, C. & Rocco, M. Solution properties of full-length integrin αIIbβ3 refined models suggest environment-dependent induction of alternative bent/extended resting states. *FEBS Journal* **277**, 3190-3202 (2010).
101. Askari, J.A., Buckley, P.A., Mould, A.P. & Humphries, M.J. Linking integrin conformation to function. *J Cell Sci* **122**, 165-170 (2009).
102. Shimaoka, M. & Springer, T.A. Therapeutic antagonists and conformational regulation of integrin function. *Nat Rev Drug Discov* **2**, 703-716 (2003).
103. Arnaout, M.A., Mahalingam, B. & Xiong, J.P. Integrin structure, allostery, and bidirectional signaling. *Annu Rev Cell Dev Biol* **21**, 381-410 (2005).
104. Partridge, A.W., Liu, S., Kim, S., Bowie, J.U. & Ginsberg, M.H. Transmembrane domain helix packing stabilizes integrin $\alpha_{IIb}\beta_3$ in the low affinity state. *J Biol Chem* **280**, 7294-7300 (2005).
105. Legate, K.R. & Fassler, R. Mechanisms that regulate adaptor binding to β-integrin cytoplasmic tails. *J Cell Sci* **122**, 187-198 (2009).

106. Bernard, E.*, et al.* Ligand Switching in Cell-Permeable Peptides: Manipulation of the α-Integrin Signature Motif. *ACS Chemical Biology* **4**, 457-471 (2009).
107. Vinogradova, O.*, et al.* A structural mechanism of integrin $\alpha_{IIb}\beta_3$ "inside-out" activation as regulated by its cytoplasmic face. *Cell* **110**, 587-597 (2002).
108. Lu, C., Takagi, J. & Springer, T.A. Association of the membrane proximal regions of the alpha and beta subunit cytoplasmic domains constrains an integrin in the inactive state. *J Biol Chem* **276**, 14642-14648 (2001).
109. Kim, M., Carman, C.V. & Springer, T.A. Bidirectional transmembrane signaling by cytoplasmic domain separation in integrins. *Science* **301**, 1720-1725 (2003).
110. Calderwood, D.A.*, et al.* Integrin beta cytoplasmic domain interactions with phosphotyrosine-binding domains: a structural prototype for diversity in integrin signaling. *Proc Natl Acad Sci U S A* **100**, 2272-2277 (2003).
111. Li, Y.F., Tang, R.H., Puan, K.J., Law, S.K. & Tan, S.M. The cytosolic protein talin induces an intermediate affinity integrin $\alpha_L\beta_2$. *J Biol Chem* **282**, 24310-24319 (2007).
112. Manevich, E., Grabovsky, V., Feigelson, S.W. & Alon, R. Talin 1 and paxillin facilitate distinct steps in rapid VLA-4-mediated adhesion strengthening to vascular cell adhesion molecule 1. *J Biol Chem* **282**, 25338-25348 (2007).
113. Lim, J.*, et al.* An essential role for talin during $\alpha_M\beta_2$-mediated phagocytosis. *Mol Biol Cell* **18**, 976-985 (2007).
114. Morgan, J.R.*, et al.* A role for talin in presynaptic function. *J Cell Biol* **167**, 43-50 (2004).
115. Monkley, S.J.*, et al.* Disruption of the talin gene arrests mouse development at the gastrulation stage. *Dev Dyn* **219**, 560-574 (2000).
116. Petrich, B.G. The antithrombotic potential of selective blockade of talin-dependent integrin $\alpha_{IIb}\beta_3$ (platelet GPIIb/IIIa) activation. *J Clin Invest* **117**, 2250-2259 (2007).
117. Wegener, K.L.*, et al.* Structural basis of integrin activation by talin. *Cell* **128**, 171-182 (2007).
118. Moser, M., Legate, K.R., Zent, R. & Fassler, R. The tail of integrins, talin, and kindlins. *Science* **324**, 895-899 (2009).
119. Harburger, D.S., Bouaouina, M. & Calderwood, D.A. Kindlin-1 and -2 directly bind the C-terminal region of beta integrin cytoplasmic tails and exert integrin-specific activation effects. *J Biol Chem* **284**, 11485-11497 (2009).
120. Honda, S.*, et al.* Integrin-linked kinase associated with integrin activation. *Blood* **113**, 5304-5313 (2009).
121. O'Toole, T.E.*, et al.* Integrin cytoplasmic domains mediate inside-out signal transduction. *J Cell Biol* **124**, 1047-1059 (1994).
122. Peter, K. & Bode, C. A deletion in the alpha subunit locks platelet integrin $\alpha_{IIb}\beta_3$ into a high affinity state. *Blood Coagul Fibrinolysis* **7**, 233-236 (1996).
123. Hughes, P.E. & Pfaff, M. Integrin affinity modulation. *Trends Cell Biol* **8**, 359-364 (1998).
124. Ma, Y.Q.*, et al.* Regulation of integrin $\alpha_{IIb}\beta_3$ activation by distinct regions of its cytoplasmic tails. *Biochemistry* **45**, 6656-6662 (2006).
125. Aylward, K., Meade, G., Ahrens, I., Devocelle, M. & Moran, N. A novel functional role for the highly conserved alpha-subunit KVGFFKR motif distinct from integrin $\alpha_{IIb}\beta_3$ activation processes. *J Thromb Haemost* **4**, 1804-1812 (2006).

126. Reddy, K.B., Smith, D.M. & Plow, E.F. Analysis of Fyn function in hemostasis and $\alpha_{IIb}\beta_3$-integrin signaling. *J Cell Sci* **121**, 1641-1648 (2008).
127. Weljie, A.M., Hwang, P.M. & Vogel, H.J. Solution structures of the cytoplasmic tail complex from platelet integrin α_{IIb}- and β_3-subunits. *Proc Natl Acad Sci U S A* **99**, 5878-5883 (2002).
128. Raab, M., Parthasarathi, L., Treumann, A., Moran, N. & Daxecker, H. Differential binding of ICln in platelets to integrin-derived activating and inhibitory peptides. *Biochem Biophys Res Comm* **392**, 258-263 (2010).
129. Liu, S.*, et al.* Binding of paxillin to α_4 integrins modifies integrin-dependent biological responses. *Nature* **402**, 676-681 (1999).
130. Hyduk, S.J., Oh, J., Xiao, H., Chen, M. & Cybulsky, M.I. Paxillin selectively associates with constitutive and chemoattractant-induced high-affinity $\alpha_4\beta_1$ integrins: implications for integrin signaling. *Blood* **104**, 2818-2824 (2004).
131. Li, S.*, et al.* Efalizumab binding to the LFA-1 α_L I domain blocks ICAM-1 binding via steric hindrance. *Proc Natl Acad Sci U S A* **106**, 4349-4354 (2009).
132. Qu, A. & Leahy, D.J. The role of the divalent cation in the structure of the I domain from the CD11a/CD18 integrin. *Structure* **4**, 931-942 (1996).
133. Shimaoka, M.*, et al.* Structures of the α_L I domain and its complex with ICAM-1 reveal a shape-shifting pathway for integrin regulation. *Cell* **112**, 99-111 (2003).
134. Gadek, T.R.*, et al.* Generation of an LFA-1 Antagonist by the Transfer of the ICAM-1 Immunoregulatory Epitope to a Small Molecule. *Science* **295**, 1086-1089 (2002).
135. Keating, S.M.*, et al.* Competition between intercellular adhesion molecule-1 and a small-molecule antagonist for a common binding site on the α_L subunit of lymphocyte function-associated antigen-1. *Protein Sci* **15**, 290-303 (2006).
136. Salas, A.*, et al.* Rolling adhesion through an extended conformation of integrin $\alpha_L\beta_2$ and relation to α I and β I-like domain interaction. *Immunity* **20**, 393-406 (2004).
137. Shimaoka, M., Salas, A., Yang, W., Weitz-Schmidt, G. & Springer, T.A. Small molecule integrin antagonists that bind to the β_2 subunit I-like domain and activate signals in one direction and block them in the other. *Immunity* **19**, 391-402 (2003).
138. Welzenbach, K., Hommel, U. & Weitz-Schmidt, G. Small molecule inhibitors induce conformational changes in the I domain and the I-like domain of lymphocyte function-associated antigen-1. Molecular insights into integrin inhibition. *J Biol Chem* **277**, 10590-10598 (2002).
139. Yang, W., Shimaoka, M., Salas, A., Takagi, J. & Springer, T.A. Intersubunit signal transmission in integrins by a receptor-like interaction with a pull spring. *Proc Natl Acad Sci U S A* **101**, 2906-2911 (2004).
140. Crump, M.P.*, et al.* Structure of an allosteric inhibitor of LFA-1 bound to the I-domain studied by crystallography, NMR, and calorimetry. *Biochemistry* **43**, 2394-2404 (2004).
141. Dodd, D.S.*, et al.* Design of LFA-1 antagonists based on a 2,3-dihydro-1H-pyrrolizin-5(7aH)-one scaffold. *Bioorgan Med Chem Lett* **17**, 1908-1911 (2007).

142. Kallen, J., *et al.* Structural basis for LFA-1 inhibition upon lovastatin binding to the CD11a I-domain. *J Mol Biol* **292**, 1-9 (1999).
143. Potin, D., *et al.* Discovery and Development of 5-[(5S,9R)-9- (4-Cyanophenyl)-3-(3,5-dichlorophenyl)-1- methyl-2,4-dioxo-1,3,7-triazaspiro[4.4]non- 7-yl-methyl]-3-thiophenecarboxylic Acid (BMS-587101)-A Small Molecule Antagonist Leukocyte Function Associated Antigen-1. *J. Med. Chem.* **49**, 6946-6949 (2006).
144. Wattanasin, S., *et al.* 1,4-Diazepane-2,5-diones as novel inhibitors of LFA-1. *Bioorg Med Chem Lett* **15**, 1217-1220 (2005).
145. Weitz-Schmidt, G., Welzenbach, K., Dawson, J. & Kallen, J. Improved lymphocyte function-associated antigen-1 (LFA-1) inhibition by statin derivatives: molecular basis determined by x-ray analysis and monitoring of LFA-1 conformational changes in vitro and ex vivo. *J Biol Chem* **279**, 46764-46771 (2004).
146. Champe, M., McIntyre, B.W. & Berman, P.W. Monoclonal antibodies that block the activity of leukocyte function-associated antigen 1 recognize three discrete epitopes in the inserted domain of CD11a. *J Biol Chem* **270**, 1388-1394 (1995).
147. McDowall, A., *et al.* The I domain of integrin leukocyte function-associated antigen-1 is involved in a conformational change leading to high affinity binding to ligand intercellular adhesion molecule 1 (ICAM-1). *J Biol Chem* **273**, 27396-27403 (1998).
148. Zhang, H., Astrof, N.S., Liu, J.H., Wang, J.H. & Shimaoka, M. Crystal structure of isoflurane bound to integrin LFA-1 supports a unified mechanism of volatile anesthetic action in the immune and central nervous systems. *FASEB J* (2009).
149. Yuki, K., *et al.* The volatile anesthetic isoflurane perturbs conformational activation of integrin LFA-1 by binding to the allosteric regulatory cavity. *FASEB J* **22**, 4109-4116 (2008).
150. Wang, Y., *et al.* Blocking LFA-1 activation with lovastatin prevents graft-versus-host disease in mouse bone marrow transplantation. *Biol Blood Marrow Transplant* **15**, 1513-1522 (2009).
151. Ahrens, I. & Peter, K. Therapeutic integrin inhibition: allosteric and activation-specific inhibition strategies may surpass the initial ligand-mimetic strategies. *Thromb Haemost* **99**, 803-804 (2008).
152. Shimaoka, M., *et al.* AL-57, a ligand-mimetic antibody to integrin LFA-1, reveals chemokine-induced affinity up-regulation in lymphocytes. *Proc Natl Acad Sci U S A* **103**, 13991-13996 (2006).
153. Artoni, A., *et al.* Integrin β3 regions controlling binding of murine mAb 7E3: Implications for the mechanism of integrin $\alpha_{IIb}\beta_3$ activation. *Proc Nat Acad Sci USA* **101**, 13114-13120 (2004).
154. Xiong, J.P., *et al.* Crystal structure of the extracellular segment of integrin $\alpha_V\beta_3$ in complex with an Arg-Gly-Asp ligand. *Science* **296**, 151-155 (2002).
155. Davenport, R.J. & Munday, J.R. Blocking α_4-integrins - A small molecule approach to treatment of multiple sclerosis. *J Neurol Sci* **274**, 27-30 (2008).
156. Gong, Y., *et al.* Synthesis and SAR of pyridazinone-substituted phenylalanine amide α_4 integrin antagonists. *Bioorg Med Chem Lett* **18**, 1331-1335 (2008).
157. Feagan, B.G., *et al.* Treatment of Ulcerative Colitis with a Humanized Antibody to the $\alpha_4\beta_7$ Integrin. *N Engl J Med* **352**, 2499-2507 (2005).

158. Feuston, B.P., *et al.* Binding model for nonpeptide antagonists of $\alpha_V\beta_3$ integrin. *J Med Chem* **45**, 5640-5648 (2002).
159. Peter, K., Schwarz, M., Nordt, T. & Bode, C. Intrinsic Activating Properties of GP IIb/IIIa Blockers. *Thromb Res* **103**, S21-S27 (2001).
160. Blue, R., Murcia, M., Karan, C., Jirouskova, M. & Coller, B.S. Application of high-throughput screening to identify a novel α_{IIb}-specific small- molecule inhibitor of $\alpha_{IIb}\beta_3$-mediated platelet interaction with fibrinogen. *Blood* **111**, 1248-1256 (2008).
161. Aga, Y., *et al.* UR-3216: a new generation oral platelet GPIIb/IIIa antagonist. *Curr Pharm Des* **10**, 1597-1601 (2004).
162. Honda, S., *et al.* Association between ligand-induced conformational changes of integrin $\alpha_{IIb}\beta_3$ and $\alpha_{IIb}\beta_3$-mediated intracellular Ca2+ signaling. *Blood* **92**, 3675-3683 (1998).
163. Ablooglu, A.J., Kang, J., Petrich, B.G., Ginsberg, M.H. & Shattil, S.J. Antithrombotic effects of targeting $\alpha_{IIb}\beta_3$ signaling in platelets. *Blood* **113**, 3585-3592 (2009).
164. Robinson, A., O'Neill, S. & Moran, N. Novel integrin-specific targets for anti-platelet therapies. *Curr Pharm Des* **10**, 1603-1609 (2004).
165. Joseph, M.C., Mark, H.G. & David, M.R. Integrin-associated proteins as potential therapeutic targets. *Immunological Reviews* **223**, 236-251 (2008).
166. Yin, H., *et al.* Computational design of peptides that target transmembrane helices. *Science* **315**, 1817-1822 (2007).
167. Kummer, C. & Ginsberg, M.H. New approaches to blockade of α_4-integrins, proven therapeutic targets in chronic inflammation. *Biochem Pharmacol* **72**, 1460-1468 (2006).
168. Kummer, C., Petrich, B.G., Rose, D.M. & Ginsberg, M.H. A Small Molecule That Inhibits the Interaction of Paxillin and α4 Integrin Inhibits Accumulation of Mononuclear Leukocytes at a Site of Inflammation. *J Biol Chem* **285**, 9462-9469 (2010).
169. Sroka, T.C., Pennington, M.E. & Cress, A.E. Synthetic D-amino acid peptide inhibits tumor cell motility on laminin-5. *Carcinogenesis* **27**, 1748-1757 (2006).
170. Cianfrocca, M.E., *et al.* Phase 1 trial of the antiangiogenic peptide ATN-161 (Ac-PHSCN-NH2), a beta integrin antagonist, in patients with solid tumours. *Br J Cancer* **94**, 1621-1626 (2006).
171. O'Neill, S., *et al.* The platelet integrin $\alpha_{IIb}\beta_3$ has an endogenous thiol isomerase activity. *J Biol Chem* **275**, 36984-36990 (2000).
172. McDonald, P.C., Fielding, A.B. & Dedhar, S. Integrin-linked kinase - essential roles in physiology and cancer biology. *J Cell Sci* **121**, 3121-3132 (2008).
173. D'Abaco, G.M. & Kaye, A.H. Integrin-linked kinase: A potential therapeutic target for the treatment of glioma. *J Clin Neurosci* **15**, 1079-1084 (2008).
174. Kalra, J., *et al.* QLT0267, a small molecule inhibitor targeting integrin-linked kinase (ILK), and docetaxel can combine to produce synergistic interactions linked to enhanced cytotoxicity, reductions in P-AKT levels, altered F-actin architecture and improved treatment outcomes in an orthotopic breast cancer model. *Breast Cancer Res* **11**, R25 (2009).
175. Younes, M.N., *et al.* Effects of the Integrin-Linked Kinase Inhibitor QLT0267 on Squamous Cell Carcinoma of the Head and Neck. *Arch Otolaryngol Head Neck Surg* **133**, 15-23 (2007).

176. Muranyi, A.L., Dedhar, S. & Hogge, D.E. Combined inhibition of integrin linked kinase and FMS-like tyrosine kinase 3 is cytotoxic to acute myeloid leukemia progenitor cells. *Exp Hematol* **37**, 450-460 (2009).
177. Funahashi, Y.*, et al.* Sulfonamide Derivative, E7820, Is a Unique Angiogenesis Inhibitor Suppressing an Expression of Integrin α2 Subunit on Endothelium. *Cancer Research* **62**, 6116-6123 (2002).
178. Aster, R.H. & Bougie, D.W. Drug-induced immune thrombocytopenia. *N Engl J Med* **357**, 580-587 (2007).
179. Calabresi, P.A.*, et al.* The incidence and significance of anti-natalizumab antibodies: results from AFFIRM and SENTINEL. *Neurology* **69**, 1391-1403 (2007).
180. Weis, S.M., Stupack, D.G. & Cheresh, D.A. Agonizing Integrin Antagonists? *Cancer Cell* **15**, 359-361 (2009).
181. Reynolds, A.R.*, et al.* Stimulation of tumor growth and angiogenesis by low concentrations of RGD-mimetic integrin inhibitors. *Nat Med* **15**, 392-400 (2009).
182. Cox, D.*, et al.* Evidence of platelet activation during treatment with a GPIIb/IIIa antagonist in patients presenting with acute coronary syndromes. *J Am Coll Cardiol* **36**, 1514-1519 (2000).
183. Davenport, R.J. & Munday, J.R. α_4-integrin antagonism -- an effective approach for the treatment of inflammatory diseases? *Drug Discovery Today* **12**, 569-576 (2007).
184. Tilley, J.W. Very late antigen-4 integrin antagonists. *Expert Opinion on Therapeutic Patents* **18**, 841-859 (2008).
185. Watterson, S.H.*, et al.* Small Molecule Antagonist of Leukocyte Function Associated Antigen-1 (LFA-1): Structure−Activity Relationships Leading to the Identification of 6-((5S,9R)-9-(4-Cyanophenyl)-3-(3,5-dichlorophenyl)-1-methyl-2,4-dioxo-1,3,7-triazaspiro[4.4]nonan-7-yl)nicotinic Acid (BMS-688521). *J Med Chem* **53**, 3814-3830 (2010).
186. Xie, C.*, et al.* Structure of an integrin with an αI domain, complement receptor type 4. *EMBO J* **29**, 666-679 (2010).
187. Humphrey, W., Dalke, A. & Schulten, K. VMD: visual molecular dynamics. *J Mol Graph* **14**, 33-38, 27-38 (1996).
188. Vinogradova, O., Haas, T., Plow, E.F. & Qin, J. A structural basis for integrin activation by the cytoplasmic tail of the α_{IIb}-subunit. *Proc Natl Acad Sci USA* **97**, 1450-1455 (2000).
189. Calderwood, D.A. Talin controls integrin activation. *Biochem Soc Trans* **32**, 434-437 (2004).
190. Naik, U.P., Patel, P.M. & Parise, L.V. Identification of a novel calcium-binding protein that interacts with the integrin α_{IIb} cytoplasmic domain. *J Biol Chem* **272**, 4651-4654 (1997).
191. Kato, A.*, et al.* Ancient ubiquitous protein 1 binds to the conserved membrane-proximal sequence of the cytoplasmic tail of the integrin alpha subunits that plays a crucial role in the inside-out signaling of $\alpha_{IIb}\beta_3$. *J Biol Chem* **277**, 28934-28941 (2002).
192. Vijayan, K.V., Liu, Y., Li, T.T. & Bray, P.F. Protein phosphatase 1 associates with the integrin α_{IIb} subunit and regulates signaling. *J Biol Chem* **279**, 33039-33042 (2004).
193. Gushiken, F.C.*, et al.* Protein phosphatase 2A negatively regulates integrin alpha IIbbeta 3 signaling. *J Biol Chem* **283**, 12862-12869 (2008).

194. Liu, Q.Y., Corjay, M., Feuerstein, G.Z. & Nambi, P. Identification and characterization of triosephosphate isomerase that specifically interacts with the integrin α_{IIb} cytoplasmic domain. *Biochem Pharmacol* **72**, 551-557 (2006).
195. Larkin, D.*, et al.* Compartmentalization regulates the interaction between the platelet integrin $\alpha_{IIb}\beta_3$ and ICln. *Br J Haematol* **144**, 580-590 (2009).
196. Brophy, T.M.*, et al.* RN181, a novel ubiquitin E3 ligase that interacts with the KVGFFKR motif of platelet integrin $\alpha_{IIb}\beta_3$. *Biochem Biophys Res Commun* **369**, 1088-1093 (2008).
197. Egbertson, M.*, et al.* Non-peptide fibrinogen receptor antagonists. 2. Optimization of a tyrosine template as a mimic for Arg-Gly-Asp. *J Med Chem* **37**, 2537-2551. (1994).
198. Nicholson, N.S.*, et al.* Orbofiban: an orally active GPIIb/IIIa platelet receptor antagonist. *Med Res Rev* **21**, 211-226 (2001).
199. Anders, R., Kleiman, J., Nicholson, N., Wazowicz, B. & Burns, D. Xemilofiban/orbofiban: insight into drug development. *Cardiovasc Drug Rev* **19**, 116-132 (2001).
200. Weller, T.*, et al.* Orally active fibrinogen receptor antagonists. 2. Amidoximes as prodrugs of amidines. *J Med Chem* **39**, 3139-3147 (1996).
201. Kelly, T.A.*, et al.* Cutting Edge: A Small Molecule Antagonist of LFA-1-Mediated Cell Adhesion. *J Immunol* **163**, 5173-5177 (1999).
202. Neidhart, W., Märki, H.P. & Mohr, P. Frontiers in Medicinal Chemistry –Joint German-Swiss Meeting on Medicinal Chemistry. *Chimia* **63**, 573-578 (2009).
203. Stragies, R.*, et al.* Design and Synthesis of a New Class of Selective Integrin $\alpha_5\beta_1$ Antagonists. *J Med Chem* **50**, 3786-3794. (2007).