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Integrin-based Therapeutics: Biological Basis, Clinical Use and New Drugs

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Abstract

Integrins are activatable adhesion and signaling molecules. Of the 24 known human integrins, three are currently targeted therapeutically by monoclonal antibodies, peptides or small molecules. The platelet αIIbβ3 integrin is targeted by Abciximab, Eptifibatide and Tirofiban, all with indications for preventing thrombotic complications after percutaneous coronary interventions. The lymphocyte α4β1 and α4β7 integrins are targeted by Natalizumab with indications in multiple sclerosis and Crohn's disease. Although efficacious, use of this antibody is limited by a rare but serious complication, progressive multifocal leukoencephalopathy. Vedolizumab is an antibody to a combinatorial epitope in α4β7 that is approved for use in patients with Crohn's disease or ulcerative colitis in the United States, Canada and Europe. Progressive multifocal leukoencephalopathy has not been observed in the clinical trials or clinical use of vedolizumab. New antibodies and small molecules targeting β7 integrins (α4β7 and αEβ7) and MAdCAM-1 are in clinical development for treatment of these inflammatory bowel diseases. Overall, integrin-based therapeutics have shown clinically significant benefits in many patients, leading to continued medical interest in the further development of novel integrin inhibitors. Of note, almost all integrin antagonists in use or in late-stage clinical trials target the ligand binding site, or the ligand itself.

Introduction

Integrins are adhesion receptors connecting cells to extracellular matrix ligands and to counter-receptors on other cells. Integrins are obligatory type I αβ heterodimers and molecular machines that undergo large conformational changes in their extracellular domains triggered by signaling molecules inside cells. This process, often referred to as inside-out signaling, is initiated by adaptor molecules that affect the position of the integrin

Competing interests

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α and β cytoplasmic tails relative to each other and to the plasma membrane. For many, if not all integrins, such conformational changes ("activation") are required to actuate their adhesive function. Current dogma holds that the ligand binding domain in resting integrins is not readily accessible to adhesive ligands.

The best-known positive regulators of integrin activation are the adaptor molecules, talin-1 [1] and the kindlins (kindlin-1, kindlin-2 and kindlin-3) [2]. Beyond adhesion, integrins are also signal transduction machines. Once activated, integrins support ligand-dependent cellular signaling, a process called outside-in signaling because it is initiated by the binding of extracellular ligands to the integrins. Outside-in signaling involves, in part, ligand-dependent clustering of integrins that brings signaling domains of integrin-proximal proteins close enough together to initiate intracellular signals. Well-known intracellular events that are dependent on integrin outside-in signaling include activation of the spleen tyrosine kinase Syk [3, 4] and Src family protein tyrosine kinases in platelets [5] and leukocytes [3], and activation of NADPH oxidase in leukocytes [6].

Given their central roles in almost all phases of human biology as well as in the pathobiology of many diseases, integrins have long been the focus of the biotechnology and pharmaceutical industries as potential therapeutic targets. The first integrin-targeted drug, Abciximab, was introduced in 1994. Currently, ClinicalTrials.gov lists 80 clinical trials regarding integrin-based therapeutic drugs, imaging agents or biomarkers.

The purpose of this Opinion piece is to provide a biological context for integrins as drug targets, to highlight integrin antagonists that have shown benefit in patients or promise in late-stage clinical trials, and to review ongoing efforts to develop new integrin-targeted drugs. We focus on mechanisms of action, on what we have learned from successes and failures, and on side effects, both expected and unexpected. Previous reviews on the subject have focused on other aspects including details of integrin structure and allosteric inhibitors [7], leukocyte integrins [8], possible targets in airway hyper-responsiveness [9] and candidate molecules in early-stage trials [10]. Not all efforts in this space have proven successful. Ten years ago, high hopes were placed in allosteric inhibitors [7], and large programs to develop such drugs were undertaken by many major pharmaceutical companies.

Integrin biology and drug development

An important lesson from past integrin drug development efforts is that successes are dependent on a combination of deep understanding of basic mechanisms of cell adhesion and unmet clinical need. All integrin antagonists on the market or in late-stage clinical trials target the ligand binding sites of integrins expressed in blood cells: leukocytes or platelets. Leukocyte and platelet integrins undergo conformational changes and "activation". Both leukocyte and platelet integrins are masters at integrin affinity regulation by inside-out signaling. For example, in leukocyte integrins, the affinity change is thought to be about 10,000-fold [11].

Nine of the 24 human integrins contain an "inserted" or I-domain that has homology to the von Willebrand factor A domain and is found in the extracellular portion of the α subunit [12]

(Figure 1). All integrins with an I-domain bind extracellular matrix ligands or counter-receptors on other cells through this domain. These integrins then undergo a conformational change providing an “internal ligand” to the β subunit I-like domain. In contrast, all integrins without an I-domain bind ligand directly in a binding pocket formed by the most N-terminal subunits of both the α and the β polypeptide chains.

The conformational change during integrin activation (Figure 2) involves extension of the α and β “legs”, rearrangement of the αβ interface in the ligand binding domain, and separation of the α and β “feet” (transmembrane domains). The αL and β2 cytoplasmic tails of LFA-1 have been shown to move apart when LFA-1 is activated [13]. This is thought to be a general process associated with integrin activation [14]. Several detailed models of integrin activation have been proposed [15, 16].

Most of the integrins without αI-domains but none of the integrins with αI-domains bind the short peptide sequence arginine-glycine-aspartic acid (RGD), first discovered by Pierschbacher and Ruoslahti [17] (Figure 1). Some of the drugs targeting platelet αIIbβ3 are based on this RGD sequence. Another short amino acid recognition sequence was identified for α4β1 integrin: ILDV in the type III CS-1 segment of fibronectin [18]. The other integrins do not bind consensus peptide sequences; the recognition site(s) in their ligands may be non-linear. A few integrins like Mac-1 (αMβ2) have also been reported to bind non-protein ligands (glycans and glycolipids), but this appears to be the exception rather than the rule. All integrins that have been targeted so far for therapeutic purposes normally bind protein ligands, and the antibody, peptide or small molecule antagonists that have made it to market all target the ligand binding site. Since integrins undergo large conformational changes during activation, allosteric inhibitors of the activation process have been proposed as drug targets [7]. Small molecules that act as allosteric inhibitors have been developed by pharmaceutical industry [19], but none of them have made it to market. It is likely that allosteric inhibitors would have limited specificity and would have effects on multiple integrins.

Integrins have several divalent cation binding sites in their extracellular domains. Under physiologic conditions, these sites are occupied by Ca^{2+} and Mg^{2+}. Mg^{2+} binding promotes the “open” or high-affinity conformation and Ca^{2+} promotes the “closed” or low-affinity conformation [20]. In vitro, absence of Ca^{2+} and presence of Mg^{2+} or (even more powerfully but artificially) Mn^{2+} can induce the high affinity conformation(s), but at physiologic levels of calcium and magnesium, integrins can exist in all three conformations shown in Figure 2. The two activated forms are thought to be transient and can revert back to the low affinity conformation after seconds to minutes.

The canonical model of integrin activation posits that integrin extension is mechanically linked to open headpiece (high affinity binding) [11]. As shown in figure 2, this would predict three conformations: bent with low affinity headpiece, extended with low affinity headpiece and extended with high affinity headpiece. Indeed, these conformations have been shown to exist on primary cells and the extended conformation with low affinity can be stabilized by certain allosteric antagonists [21]. This conformation appears to support neutrophil rolling, but not firm adhesion [22–24]. Although a large number of allosteric antagonists have been made

that effectively inhibit either extension or the high affinity conformation [7, 19, 25], these have not been successful as systemic therapeutics. We speculate that either the specificity of these molecules was insufficient, i.e., each blocker would block multiple integrins, or unexpected systemic toxicity may have occurred. Alternatively, or in addition, the proposed conformational changes during activation, which have mainly been determined for αVβ3, αIIbβ3 and the β2 integrins, may not apply directly to α4β1 and α4β7 integrins so that the allosteric inhibitors would not work in some therapeutically relevant integrins. A few allosteric inhibitors for α4β1 have been described in preclinical studies [26, 27], but there is no evidence that any have been developed further or gone into clinical trials.

The clinically successful integrin drugs target αIIbβ3 (table 1), α4integrins (table 2) and α4β7 (table 3). Therefore, the remainder of this opinion piece is organized along these targets with a focus on new drugs.

Platelet integrins

αIIbβ3

Inherited deficiency or dysfunction of αIIbβ3[28] causes a rare but serious bleeding disorder, Glanzmann thrombasthenia, due to the inability of activated platelets to aggregate in a ligand-dependent manner. Platelets also express four other integrins, including α2β1, which binds collagen, but its signaling function in stimulating platelet aggregation and secretion is minor compared to that of the (non-integrin) collagen receptor GPVI [29]. αIIbβ3 has some affinity for immobilized fibrinogen even without deliberate platelet activation. When platelets are fully activated, αIIbβ3 can bind soluble fibrinogen, von Willebrand factor, fibronectin and vitronectin [28, 30] in a manner that depends on the presence of one or more of the RGD sequences in the ligands, or in the case of fibrinogen, on the carboxy-terminus of the γ chain [31, 32]. The dimeric fibrinogen molecule mediates platelet aggregation by serving as a bridge between, αIIbβ3 receptors on adjacent platelets.

Inside-out activation of αIIbβ3 is very well studied [14]. A key element is binding of talin-1 to a membrane-proximal region and an NPXY motif in the β3 cytoplasmic domain [1]. Identifying the gene responsible for a rare inherited bleeding disorder in which αIIbβ3 cannot be activated has led to the recognition that kindlin-3 is also required for this process [3334]. The precise mechanism(s) by which kindlin-3 influences integrin activation is incompletely understood but appears to involve, at least in part, kindlin-3 interaction with the C-terminal region of the β3 cytoplasmic domain and clustering of αIIbβ3 heterodimers into oligomers [35]. All reported patients with a null mutation in kindlin-3 also exhibit defective activation of their leukocyte integrins [34], resulting in recurrent infections. The syndrome is therefore known as leukocyte adhesion deficiency (LAD) type III (or LAD type I variant).

After blood cell development, αIIbβ3 is expressed exclusively in megakaryocytes and platelets. This restricted expression and the obligatory requirement for ligand binding to αIIbβ3 in platelet aggregation during hemostasis and thrombosis led investigators to consider this integrin as a potential therapeutic target for development of anti-platelet, anti-thrombotic drugs [28, 30]. Abciximab, the Fab fragment of a chimeric mouse-human

monoclonal antibody to αIIbβ3, was the first integrin antagonist in clinical medicine [36]. Two additional parenteral, non-antibody αIIbβ3 antagonists, Eptifibatide [37] and Tirofiban [38], quickly followed for similar indications, and all three drugs work by directly blocking ligand binding to αIIbβ3. As knowledge pertaining to mechanisms of αIIbβ3 signaling has increased in recent years, therapeutic blockade of specific intracellular facets of inside-out and/or outside-in αIIbβ3 signaling remains an appealing, if only theoretical, possibility [39].

αVβ3

In contrast to αIIbβ3, αVβ3 is more widely expressed in tissues, particularly in proliferative endothelial cells, where it has been implicated in aspects of angiogenesis, and in vascular smooth muscle cells, monocyte/macrophages and some tumor cells [40]. αVβ3 can interact with many of the same RGD-containing adhesive proteins as αIIbβ3, but with different affinities, and it can interact with a number of non-RGD-containing proteins in the extracellular matrix. Despite substantial efforts at development by the pharmaceutical industry [10], an αV antagonist, Cilengitide, blocks the binding of vitronectin to αVβ3 but has not shown efficacy in clinical trials aimed at limiting tumor angiogenesis and progression in patients with glioblastoma [41]. Its failure in this context may be due to complexities in the dose- and timing-dependent mechanism of action of Cilengitide administration as shown in mouse models [42] as well as the inherent difficulties of treating a notoriously resistant neoplasm with a single targeted drug [43].

αIIbβ3 in cardiovascular medicine

Abciximab[36, 44] binds to αIIbβ3 with nanomolar affinity and inhibits the binding of fibrinogen, von Willebrand factor and other RGD-containing adhesive ligands to human αIIbβ3. As a result, Abciximab blocks agonist-induced aggregation of human platelets as well as downstream platelet responses dependent on aggregation [28, 44]. The epitope for this antibody is in the specificity-determining loop of β3, close to the β3 “MIDAS” metal ion-dependent adhesion site [45]. In addition to αIIbβ3, Abciximab has been reported to bind to αVβ3 and, to a lesser extent, αMβ2, and to inhibit αVβ3-dependent endothelial cell spreading in vitro [46, 47]. The role, if any, of αVβ3 or αMβ2 binding by Abciximab in its anti-thrombotic efficacy in humans is unclear. Abciximab is indicated for use with heparin and aspirin as an adjunct for the prevention of cardiac ischemic complications, either in patients undergoing PCI or in patients with unstable angina not responding to conventional medical therapy in whom PCI is planned within 24 hours (table 1).

Eptifibatide is a disulfide-linked, cyclic heptapeptide containing a 1-mercaptopropionyl residue and is based on the amino acid sequence Lys-Gly-Asp (KGD) in the snake venom, barbourin.[37, 44, 48] Eptifibatide is highly selective for αIIbβ3 and binds to the integrin at the ligand-binding pocket in a divalent cation-sensitive manner to reversibly inhibit adhesive ligand binding and platelet aggregation. Eptifibatide is indicated for patients with non-ST elevation myocardial infarction (NSTEMI), including those who are to be managed medically and those undergoing PCI (table 1).

Tirofiban is a highly selective small molecule (N(butylsulfonyl)-O-(4-(4-piperidinyl)butyl)-L-tyrosine monohydrochloride monohydrate) inhibitor of αIIbβ3 that was approved for

human use in 1999 [38, 44]. Like Eptifibatide, it is highly selective for αIIbβ3 and blocks ADP-induced platelet aggregation. Tirofiban is indicated to reduce the rate of thrombotic cardiovascular events in patients with NSTEMI who are undergoing PCI (table 1).

Adverse events with αIIbβ3 antagonists

Unsurprisingly, the most serious side effects common to all parenteral αIIbβ3 antagonists are bleeding and thrombocytopenia. The main underlying mechanism is immunological but appears to vary in detail depending on the drug [49]. In the case of Abciximab, thrombocytopenia appears to be caused in most cases by the development of antibodies to murine sequences in the chimeric Fab fragment. In individuals receiving Eptifibatide or Tirofiban, thrombocytopenia typically occurs during the drug infusion and appears to be caused in most cases by antibodies to extracellular epitopes in αIIbβ3 that are exposed by binding of the drug. These antibodies may be pre-formed and naturally occurring in these individuals [49, 50]. When thrombocytopenia occurs with any αIIbβ3 antagonist, the drug must be discontinued and platelet transfusion given if clinically indicated.

Impact of the availability of newer anti-platelet and anti-thrombotic drugs

Currently, no active clinical trials testing new inhibitors of αIIbβ3 are listed in clinicaltrials.gov. This is owing largely to the introduction of P2Y12 and PAR1 thrombin receptor inhibitors, which has led to a reduction in the market for αIIbβ3 integrin antagonists. In ISAR-REACT-2, Abciximab reduced the risk of adverse events in patients with NSTEMI undergoing PCI, even after optimal pre-treatment with 600 mg of clopidogrel [51]. However, with the increased availability of efficacious newer parenteral anticoagulants (e.g., bivalirudin) and newer $P2Y_{12}$ receptor antagonists that are more potent and/or more rapdily acting than clopidogrel (e.g., cangrelor and the orally bioavailable prasugrel and ticagrelor), the use of αIIbβ3 antagonists has decreased [52]. Nonetheless, the αIIbβ3 antagonists appropriately remain in the cardiologist's arsenal, particularly for use in high-risk individuals undergoing PCI, including those in whom the use of $P2Y_{12}$ antagonists might have been delayed or are likely to be relatively ineffective.

Failure of oral αIIbβ3 antagonists

Given the efficacy of parenteral αIIbβ3 antagonists in the setting of acute coronary syndromes (which include NSTEMI, ST-elevation myocardial infarction and unstable angina) and PCI, several oral agents selective for αIIbβ3 were developed and tested in phase III trials. These studies showed that the use of the oral agents was associated with excess bleeding and mortality, the latter primarily due to cardiovascular events [28, 30, 53]. The reasons for this failure are still debated, although three issues seem germane [54]. First, the successful studies with parenteral αIIbβ3 antagonists employed drug doses and infusion schemes that resulted in high-grade, continued occupancy of αIIbβ3 (>80%) during the treatment phase. This degree and continuity of receptor inhibition might be very hard to maintain with an oral αIIbβ3 antagonist, such that periods of αIIbβ3 availability to fibrinogen and other adhesive ligands would be expected to occur, enabling aggregation of activated platelets at sites of vascular pathology. Second, most oral αIIbβ3 antagonists are expected to change the conformation of the receptor upon binding, possibly leading to an unintended partial agonist effect on platelets [55]. The failure of oral αIIbβ3 inhibitors is a

good example of how perfectly sound mechanistic reasoning (these drugs should have worked) paired with unfavorable pharmacokinetics and unexpected allosteric effects (conformation change) can result in adverse outcomes that were completely unpredictable until the clinical trial data became available.

Future prospects for αIIbβ3 antagonists

Although αIIbβ3 is a proven therapeutic target, the initial wave of failures of oral αIIbβ3 antagonists effectively eliminated pharmaceutical companies from this development space. Recently, however, Coller and colleagues identified an αIIbβ3-selective compound from a high-throughput drug screen that does not induce a detectable conformational change in αIIbβ3 when it binds to the RGD binding site in the receptor. Subsequent structure-based drug design and development of a water-soluble congener, RUC-4, demonstrated a sub-micromolar IC_{50} for ADP-induced platelet aggregation, both in vitro and after intramuscular administration to non-human primates [56]. RUC-4 may be further evaluated for potential administration to patients with acute coronary syndromes in the pre-hospital setting, because the drug could be available in a formulation for intramuscular administration, a clear advantage over intravenous αIIbβ3 antagonists in an ambulance setting.

Leukocyte integrins

Six integrins are expressed exclusively in leukocytes: LFA-1 (αLβ2), Mac-1 (αMβ2), p150, 95 (αxβ2), αdβ2, α4β7 and αEβ7. Five of these contain αI–domains (Figure 1). LFA-1 (αLβ2) has been shown to undergo very large conformational changes secondary to inside-out signaling as revealed by cryo-electron microscopy [57] and crystallography[58], which is associated with ~10,000 fold increases in ligand binding affinity[11]. Patients with Leukocyte Adhesion Deficiency Type I (LAD-I) have hypomorphic or null mutations in the β2 chain, also known as CD18, common to αLβ2, αMβ2, αxβ2 and αdβ2 and exhibit mild to severe inflammatory defects [59]. There are no known human genetic defects in the four individual α chains or in either of the β7 integrins.

Leukocyte integrins play a prominent role in inflammation and immunity. Specifically, the β2 integrin LFA-1 is required for the formation of the immunological synapse [60]. The association with lymphocyte function is actually what gave it its name. αL knockout mice have reduced lymphocyte numbers in their secondary lymphoid organs and a mild defect in inflammation [61]. Mac-1 (αMβ2) plays a major role in host defense, especially against bacterial and fungal infections. Mac-1 is also known as complement receptor CR3 and is a major molecule recognizing complement C3bi-opsonized particles. αM knockout mice have a defect in neutrophil apoptosis [62] and reduced proteinuria in a mouse model of immune complex-induced kidney disease [63]. A single nucleotide polymorphism in the human *ITGAM* gene encoding the αM subunit of αMβ2 is highly associated with lupus erythemaosus [64, 65]. αxβ2 is also known as CR4. The knockout mouse has no spontaneous phenotype, but was shown to play a role in a model of atherosclerosis [66]. Combined knockout of all four β2 integrins by targeting the β2 subunit *Itgb2* results in a very severe inflammatory disease with high neutrophil numbers, spontanmeous infections and an inability of neutrophils to assemble the NADPH oxidase [6].

Among the leukocyte-specific integrins, both α4β7 and αEβ7 direct lymphocyte trafficking to the intestinal tissues. α4β7 is the major and defining determinant of gut-homing lymphocytes [67, 68]. αEβ7 binds to E-cadherin and places lymphocytes known as intraepithelial lymphocytes (IEL) near or inside the epithelial monolayer lining the intestine. α4β1 was originally identified on lymphocytes activated for extended periods and named very late antigen-4 (VLA-4) [69]. It binds VCAM-1 [70] and other ligands on endothelial cells and is involved in adhesion of effector, effector-memory and central memory cells to many, if not all, inflamed organs.

The rationale for targeting leukocyte integrins is to modulate inflammation. An early major observation in the field was that antibodies to α4β1 can cure EAE, a mouse model of multiple sclerosis, which spawned the clinical development of α4 antagonists (see below and table 2). Mice lacking all four β2 integrins or individual β2 integrins, or mice in which β2 integrins are blocked by antibodies are protected in many models of ischemia and reperfusion [71, 72].

α4β7 is targeted by the antibody Vedolizumab, which has recently proven useful in the treatment of inflammatory bowel diseases, as well as by another antibody, AMG181. α4β7 does not contain an I-domain and binds predominantly to MAdCAM-1, which is expressed on endothelial cells in tissues of the gastrointestinal tract [73]. The α4β7 ligand MAdCAM-1 is the target of a new antibody to treat inflammatory bowel disease (see below).

αEβ7 binds E-cadherin and is thought to be involved in localizing leukocytes to gut epithelial cells. An antibody targeting β7 is in late-stage clinical development for inflammatory bowel diseases (see below). A seventh leukocyte integrin, α4β1, is expressed on monocytes and lymphocytes, but, unlike the other six, is also expressed on many other cells. It binds both a splice variant of fibronectin containing the peptide sequence ILDV as well as vascular endothelial cell adhesion molecule-1, VCAM-1 [70], which supports slow rolling, adhesion and transmigration as well as pro-inflammatory signaling into the endothelial cells. α4β1 has no I-domain and is targeted by the antibody Natalizumab, with indications in multiple sclerosis (MS) and Crohn's disease (CD) (see below).

Targeting leukocyte integrins

Targeting leukocyte integrins has proven applications in diseases such as multiple sclerosis and the inflammatory bowel diseases Crohn's disease (CD) and ulcerative colitis (UC). Four leukocyte integrins, αLβ2, α4β1, α4β7 and αEβ7 have been targeted by monoclonal antibodies in patients. αL is the target of efalizumab, which was previously on the market for psoriasis but was withdrawn in 2009 because of association with a fatal brain infection, progressive multifocal leukoencephalopathy (PML, see box 1). A topical LFA-1 inhibitor, lifitegrast, recently successfully completed a phase III trial, the SONATA study, examining lifitegrast ophthalmic solution in patients with dry eye (see Further information) Lifitegrast is a small-molecule integrin antagonist designed to reduce inflammation. It binds to αLβ2

Further information
http://www.medscape.com/viewarticle/843968

integrin (LFA-1) and blocks the interaction of LFA-1 with its cognate ligand ICAM-1. ICAM-1 is over-expressed in corneal and conjunctival tissues in dry eye disease. In the SONATA study, adverse events occurred in 53.6% of patients in the lifitegrast group and 32.4% of patients in the placebo group, but there were no serious ocular adverse events or systemic toxicity and discontinuation due to adverse events was infrequent. The FDA granted lifitegrast priority review status on April 9, 2015.

The first widely successful drug targeting leukocyte integrins was natalizumab with indications in multiple sclerosis and Crohn's disease. All integrin-targeting drugs clinically approved for IBD are humanized monoclonal antibodies that target α4 integrins or the α4β7 heterodimer (Figure 3). Antibodies that target the β7 integrin subunit and the MAdCAM-1 ligand are currently in clinical trials.

Targeting α4 integrins in MS

α4 can pair with β1 to form VLA-4 or with β7 to form α4β7 (Figures 1, 3). Therefore, drugs targeting α4 effectively inhibit two integrins, α4β1 and α4β7 (Figure 3). Natalizumab is a recombinant humanized IgG4κ monoclonal antibody that binds to α4 integrins and blocks the binding of physiological ligands. α4β1 integrin binds VCAM-1, which is expressed on inflamed endothelial cells, macrophages and other cells, and alternatively spliced fibronectin, an extracellular matrix component. α4β7 binds MAdCAM-1, expressed on intestinal endothelial cells (Figure 3). These properties were demonstrated *in vitro*, as Natalizumab effectively prevented adhesion of human Jurkat cells that expressed α4β1 to purified recombinant VCAM-1 and of RPMI-8866 cells that expressed α4β7 to recombinant MAdCAM-1. These data were complemented by *in vivo* studies of experimental allergic encephalomyelitis (EAE) in rodents. This model is mediated by T-lymphocytes that infiltrate regions of the central nervous system via α4β1/VCAM-1-mediated migration. A monoclonal α4 antibody prevented leukocytes from crossing the blood-brain barrier and prevented the development of neurological manifestations of EAE and reversed established disease [74]. In all, these results provided a direct proof for the efficacy of natalizumab-like antibodies as an anti-adhesion drug in animal models.

Natalizumab is effective in treating patients with multiple sclerosis (clinical trials summarized in table 2). Approximately 6% of individuals receiving Natalizumab have been found to develop efficacy-reducing antibodies to the drug [75]. Natalizumab is approved in the United States and the European Union as monotherapy for the treatment of highly active relapsing and remitting MS in spite of prior treatments. The unexpected development of PML (box 1) in patients treated with natalizumab triggered a voluntary withdrawal of the drug from the market in February 2005. Remarkably, MS patient advocacy groups lobbied the US Food and Drug Administration (FDA) to make Natalizumab available again, because the benefits were so significant. Natalizumab returned in July 2006 under a strict TYSABRI Outreach: Unified Commitment to Health (TOUCH) monitoring program. More than 100,000 MS patients have been treated with Natalizumab.

Targeting α4 integrins in IBD

Natalizumab is also approved by the FDA as a remission-inductive and maintenance-sustaining therapy for CD. The success of natalizumab in patients with CD (see table 2 for clinical trials) provided a strong incentive to develop more specific drugs targeting α4 integrins in the intestinal tract. This was achieved by targeting α4β7, β7 and MAdCAM-1.

Targeting α4β7 in IBD

Pre-clinical studies in cotton top tamarins with spontaneous colitis provided evidence for an anti-inflammatory effect of α4β7 blockade in experimental intestinal inflammation [76, 77]. Based on these results, Vedolizumab (previously known as MLN-02, LDP-02, MLN0002, brand name Entyvio), a humanized IgG-1 monoclonal antibody, was developed. This antibody blocks binding of MAdCAM-1 to α4β7 integrin by binding to the integrin heterodimer The clinical trials of Vedolizumab in CD and UC are summarized in table 3 and the drug is approved by the FDA for the treatment of UC and CD.

As PML risk is the limiting factor for the use of Natalizumab, Vedolizumab has effectively replaced Natalizumab in clinical practice for CD and UC. At the same time, these encouraging results have spawned studies into new indications and new drugs in the β7 integrin space. Potential new indications for Vedolizumab include primary sclerosing cholangitis, based on the expression of MAdCAM-1 in chronically inflamed liver [78]. Refractory pouchitis is another potential new indication. No published clinical data are available at this time.

New leukocyte integrin antagonists for IBD

AMG 181

AMG 181 is a human monoclonal antibody (IgG2) against the α4β7 integrin heterodimer. AMG 181 is conceptually similar to vedolizumab, because it also binds a combinatorial epitope, which means it binds neither α4 nor β7 in isolation. The drug was generated at Amgen by expression in CHO cells [79] and is administered via the subcutaneous route. Safety data was published in 2014 [80] . No cases of PML have been observed. There is no published data regarding similarities or differences between AMG 181 and Vedolizumab in terms of their binding site. One study, NCT01290042, comparing four escalating doses of AMG 181 administered as multiple doses in healthy subjects and in subjects with active UC has been completed but not yet published. A phase I study (NCT01164904) in healthy volunteers and patients with UC was terminated. The reason for termination is not available. Two phase 2 studies in UC (NCT01694485) and CD (NCT01696396) are listed as active at http://clinicaltrials.gov.

Etrolizumab

Etrolizumab is a humanized IgG1 monoclonal antibody that is directed against the β7 integrin subunit, thus targeting both αEβ7 and the α4β7 (Figure 3) and blocking their interactions with MAdCAM-1 and E-cadherin, respectively. It is not known whether additional aspects of immunology would be targeted by etrolizumab, but theoretically it should target intestinal intraepithelial lymphocytes (IELs), which express αEβ7. A subset of

dendritic cells that produce anti-inflammatory retinoic acid and support the development of regulatory T cells also express αEβ7 [81] and thus might be targeted by Etrolizumab. Pre-clinical studies showed that Etrolizumab effectively inhibits migration of T-cells to mucosal sites, without affecting their homing to non-mucosal tissue [82]. In a randomized, phase I study on the use of Etrolizumab (PRO145223) in moderate to severe UC, the drug was safe and well tolerated [83]. Serious adverse effects included exacerbation of UC and impaired wound healing in two patients who underwent colectomy. There was a decrease in "availability" of β7 receptors on target CD4+ lymphocytes, suggesting that Etrolizumab administration might decrease the number of lymphocytes homing to the gut.

The results of a double-blind, placebo-controlled randomized, phase 2 study on the use of etrolizumab in patients with UC were recently reported [84]. Etrolizumab was safe and well tolerated, and no serious opportunistic infections were reported. In that study, Etrolizumab was more likely than placebo to lead to clinical remission at week 10, however the high dose did not provide added benefit. Interestingly, anti-TNF non-responders fared worse than anti-TNF-naïve. Although the biologic basis for this observation is unknown at the present time, we may speculate that the anti-TNF non-responder population is enriched for those with the most therapy-resistant diseaseThe mechanisms of non-response are not clearly understood, but have tentatively been attributed to anti-drug antibodies or pharmacokinetics.

The study additionally found increased CD4+β_7^+ T cells in peripheral blood, which consistent with the hypothesis that Etrolizumab interferes with effector T cell recruitment into the intestine. However, they found no change in β_7, β_1 or α_E mRNA levels in intestinal biopsies, which would have further supported this possibility. Interestingly, when the investigators looked at subsets of patients that were either α_E^{high} or α_E^{low} they observed that most patients with clinical remission at day one were α_E^{high}. This is interesting as this $\alpha_E\beta_7$ heterodimer has been more commonly associated with tolerogenic/immunoregulatory cells in populations of both T cells and dendritic cells. We could speculate that different doses of this drug might result in distinct levels of the drug in tissues that interfere with the $\alpha_4\beta_7$ heterodimer at one level and with the $\alpha_E\beta_7$ heterodimer at another level. This could have consequences that would not be expected based on the clinical experience with vedolizumab, which does not bind to the $\alpha_E\beta_7$ heterodimer. $\alpha_E\beta_7$ has been most recently implicated on tissue resident memory cells (TRM cells), [85] however, both the functional role of $\alpha_E\beta_7$ in TRM cells and the role of TRM cells in chronic inflammatory processes are poorly understood.

AJM 300

AJM 300 is an oral compound that acts as an antagonist of α4 integrins. All information regarding molecular structure and binding site remains unpublished by Ajinomoto Inc. Kawasaki Japan. Several studies have reported the efficacy of this small molecule in animal models of IBD. A manuscript was submitted and later withdrawn as the authors did not comply with journal requirements for publishing the molecular structure [86]. Similarly, the results of a randomized, double-blind, placebo controlled trial in Japanese patients with active CD was presented during the Digestive Diseases Week meeting in 2009 [87] AJM 300 was safe and well tolerated and showed a statistically significant improvement in clinical

response rate in patients with moderately active UC. However, this drug is likely to cause PML at a rate similar to natalizumab which would be an unacceptable risk in UC patients. Further evaluation of the potential safety of AJM 300 in IBD will be required.

Targeting integrin ligands

Since targeting α4β7 has been so successful, MAdCAM-1 became an obvious target. MAdCAM-1 is normally expressed in the mesentric lymph node and Peyers patches, but becomes more widely expressed in other venules of the intestinal wall during inflammation. Many of the endothelial ligands for integrins share structural and genetic features with immunoglobulin molecules; they contain at least one immunoglobulin domain, comprising two β-pleated sheets held together by a disulfide bond. Among the many members of the immunoglobulin superfamily, several have established pathogenetic roles in IBD. Intercellular adhesion molecule-1 (ICAM-1, CD54), Vascular Cell Adhesion Molecule-1 (VCAM-1, CD106) and MAdCAM-1 are all known to be involved in IBD, but only MAdCAM-1 is gut-specific.

MAdCAM-1: the endothelial $\alpha_4\beta_7$ integrin ligand

MAdCAM-1 levels are increased in the colon of animal models of colitis [88] and in humans with IBD the number of intestinal mucosal vessels that stain positive for MAdCAM-1 is increased[89]. TNF-α and IL-1 are abundant in areas of active CD or UC and have been shown to upregulate MAdCAM-1 expression in the intestine, colon and MLN [88, 90]. MAdCAM-1 is detected at extra-intestinal sites, such as the joints, eyes, skin and liver [91]. As these organs are frequently affected in patients with IBD, the aberrant expression of this gut-homing molecule may attract pathogenic cells and induce extra-intestinal inflammation. MAdCAM-1 expression is increased on inflamed venules in other chronic inflammatory conditions such as diabetes, primary sclerosing cholangitis, and cirrhosis [92].

PF-00547659 is a fully human IgG2K monoclonal antibody that binds specifically to human MAdCAM-1. In functional assays the drug blocked the adhesion of cells expressing $\alpha_4\beta_7$ integrin to MAdCAM-1 [93]. The results of the phase II TURANDOT study were presented at the Digestive Diseases Week Meeting 2015 [94] The primary endpoint of clinical remission was significantly greater in the three lowest dose groups compared with placebo. The secondary endpoint of mucosal healing was significantly greater in the 22.5 mg and 75 mg dose groups compared with placebo, while response was greater for the 22.5 mg and 225 mg groups. This study was not powered to compare the different doses of drug, and so it is unclear whether the observed lower remission rates in the 225 mg group was statistical play of chance, or whether there is a biologic basis for this observation. There was no evidence of increased infections in mucosal tissues (gastrointestinal, nasal, spleen, bladder, uterus and lung) and no cases of progressive multifocal leukoencephalopathy (PML) were observed. Of note, a consistent finding for all endpoints was that the second-lowest of the four doses tested was the most effective.

Another anti-MAdCAM-1 antibody, PF-00547659, has been investigated for the treatment of Crohn's Disease. The results of a randomized, multicenter double-blind, placebo-controlled study were presented at the Digestive Diseases week 2015 [95] Although the

primary endpoint disease score was not significantly different between any of PF-00547659 doses and placebo, remission at week 12 appeared to be substantially higher in those patients with a median baseline CRP level >18. The primary endpoint was not met due to a high placebo response. However, PF-00547659 was pharmacologically active as shown by a dose-related increase in circulating β_7+ T lymphocytes and a sustained dose-related decrease in soluble MAdCAM-1 in the blood MAdCAM-1 levels remained low during the study in patients who received drug. Circulating β_7 CD4+ central memory T-lymphocytes increased at weeks 8 and 12 in patients treated with PF-00547659 in a dose-dependent manner. This suggests that MAdCAM-1 is relevant in rolling and adhesion of α4β7+ lymphocytes in these patients and blocking MAdCAM-1 releases these lymphocytes into the circulation. Interestingly, higher rates of remission in patients with high CRP levels were also observed in the Natalizumab trials, completed nearly a decade ago. Objective outcomes other than disease score will be needed for future CD trials. Endoscopic, histologic and magnetic resonance imaging outcomes are being extensively discussed in the field.

Other ligands that could be targeted include ICAM-1, a ligand for LFA-1 and Mac-1, and VCAM-1, a ligand for α4β1. ICAM-1 was targeted by mAb RR6.5 (Enlimomab, Boehringer Ingelheim) early on [96], but this agent was not effective in a clinical trial of 625 patients with ischemic stroke that were treated within 6 hours of stroke onset [97]. VCAM-1 is expressed broadly on endothelial cells but also in macrophages. Notably, as VCAM-1 is the main ligand of α4β1, blocking VCAM-1 would be expected to have a PML liability.

The experience with integrin-targeted drugs in cancer is limited to αVβ3, which is associated with angiogenic endothelium in some cancers but not others. Theoretically, targeting β2 integrins could limit the infiltration of myeloid-derived suppressor cells (MDSCs), which are known to enhance tumor growth and metastasis. However, the lessons from β2 integrin null mice and people (LAD-I) suggest that severe host defense and inflammation issues would arise. The fact that only molecules targeting the ligand binding site or the ligand have been successful suggests that we do not know enough about integrin conformation change during activation to successfully construct allosteric inhibitors. Theoretically, it might be possible to target talin-1, an adaptor molecule that regulates the affinity of β2 and β3 integrins. However, knockout of talin-1 in mice is lethal [98], suggesting that talin-1 has other important functions. Another adaptor molecule involved in integrin-mediated leukocyte and platelet adhesion is kindlin-3. Kindlin-3 knockout mice [99] and people (LAD-III) [34] have severe bleeding and an infectious diathesis. Once integrins bind their ligands, outside-in signaling ensues [4,100], and there are known drug targets in this signaling pathway, for example Syk [101] and Src kinases [100]. Targeting these tyrosine kinases cannot be expected to be specific for integrin signaling, because they are involved in Fc receptor and B cell receptor signaling as well.

Conclusions

The development and demonstrated efficacy of integrin antagonists are a prime example of translational medicine whereby a deep fundamental knowledge of integrin biology has informed the design of antibody, peptide and small molecule drugs that were successful in Phase III clinical trials. In turn, the results and adverse events observed in these trials have informed our understanding of pathophysiology. Integrin-targeting drugs have found four

main indications: thrombosis prevention after PCI (αIIbβ3 integrin), ulcerative colitis and Crohn's disease (α4β7 integrin) and multiple sclerosis (α4β7 and α4β1 integrins). All approved drugs prevent the target integrin from binding its ligand(s). With the exception of Natalizumab, which carries a significant risk for PML, the other integrin-targeting drugs have proven remarkably safe and effective. Hundreds of thousands of patients have benefited from these drugs. In the future, one can anticipate expanded indications for existing integrin antagonists, particularly those in the α4 and β7 space. Antibody drugs targeting integrin ligands are emerging, as exemplified by the antibodies to MAdCAM-1.

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Box 1: Progressive Multifocal Leukoencephalopathy: A Major Complication

The widespread use of anti-integrin monoclonal antibodies for the treatment of IBD, multiple sclerosis or psoriasis has been hampered by the occurrence of a rare but potentially fatal complication, PML [102]. This condition is the result of reactivation of a polyoma virus, which is designated John Cunningham (JC) virus. The risk for developing PML after treatment with Natalizumab has been estimated to be approximately 2:1000 for patients treated for more than two years. Until 2009, four cases were described within a cohort of 6000 patients that had received Efalizumab for psoriasis. The unexpected development of PML in patients treated with Natalizumab triggered its voluntary withdrawal from the market in February 2005 (it returned in July 2006 under TOUCH monitoring) and of Efalizumab in 2009. PML appears to be a true drug-effect, as neither MS, CD nor psoriasis, per se, have been associated with PML. There is no known treatment, prevention or cure for PML. The infection usually leads to death or severe disability.

The pathogenesis of PML in patients receiving Natalizumab is largely unknown. Nevertheless, it may be primarily associated with the blockade of α4β1/VCAM-1 interactions by Natalizumab. This may result in the blockade of migration of JCV-specific lymphocytes to the central nervous system, including cytolytic T-lymphocytes, which have been associated with increased survival from PML [103]. Alternative pathogenic mechanisms may also participate, such as mobilization of JC-infected pre-B-cells from the bone marrow due to α4β1 blockade [104]. If blockade of α4β1/VCAM-1 interactions is mainly responsible for PML development in the central nervous system, then it should be expected that selective blockade of α4β7 may not be associated with this complication. Indeed, there have been no cases of PML to date in patients treated with the specific anti- α4β7 antibody vedolizumab.

It is not clear whether PML is a “class” adverse effect. Cases of PML have also been reported in patients receiving rituximab [105], an anti-CD20 monoclonal antibody that primarily targets B-cells. Nevertheless, a causal association between this drug and PML cannot be directly established, since some of the conditions for which rituximab was administered (lymphoproliferative disorders, systemic lupus erythematosus and rheumatoid arthritis) may inherently increase the risk for developing PML. The frequency of PML in patients who are negative for JC virus (around 50% of patients) is near zero, thus it is possible that anti-integrin antibodies might be safely used in subsets of JC-seronegative patients.

The association between anti-integrin monoclonal antibodies and PML has been a significant impediment for their widespread use in IBD clinical practice. Current strategies to overcome this problem have focused on the careful pre-testing for JC virus antibodies in treatment candidates and thorough monitoring of JC serologic conversion in actively treated patients.

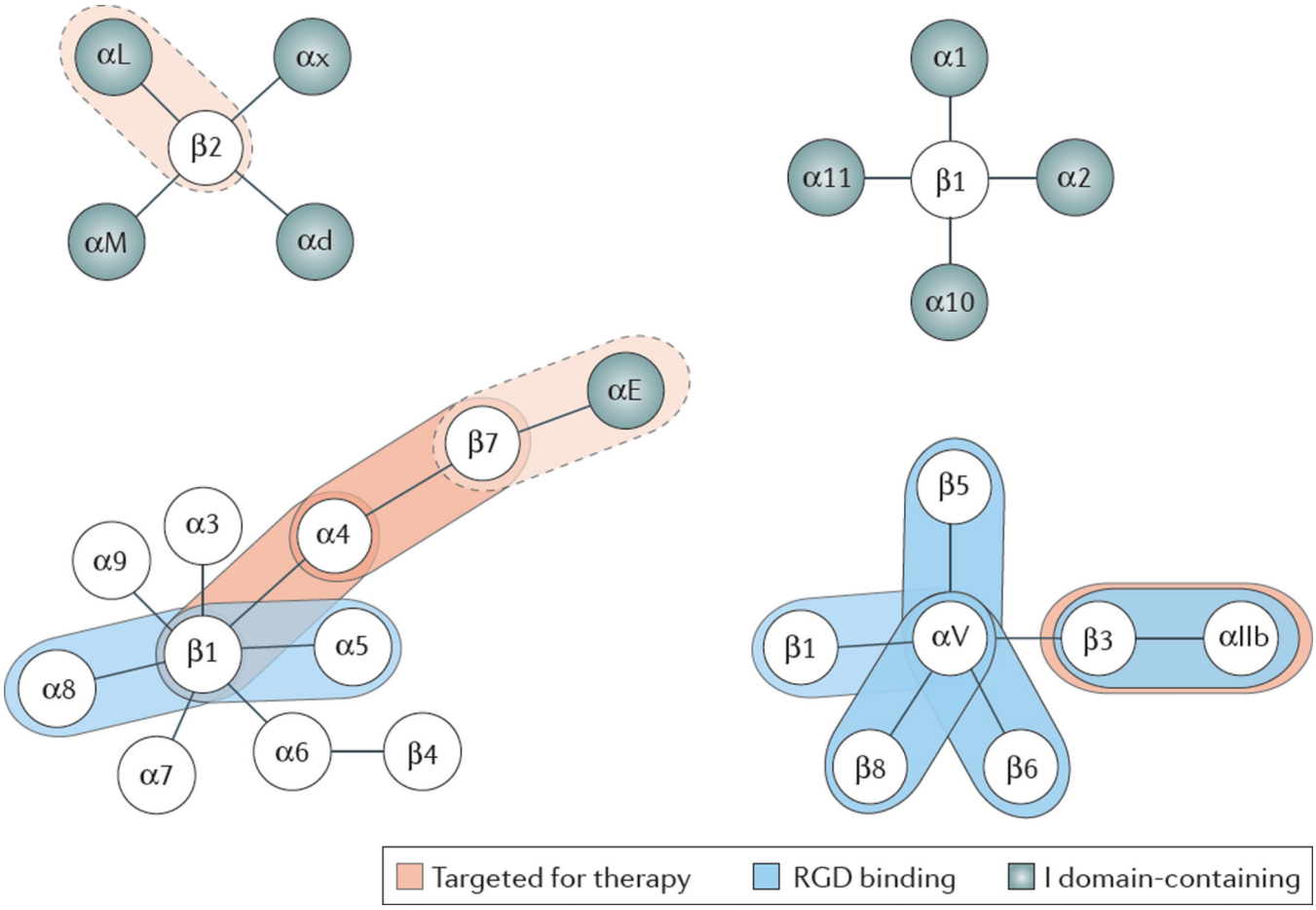
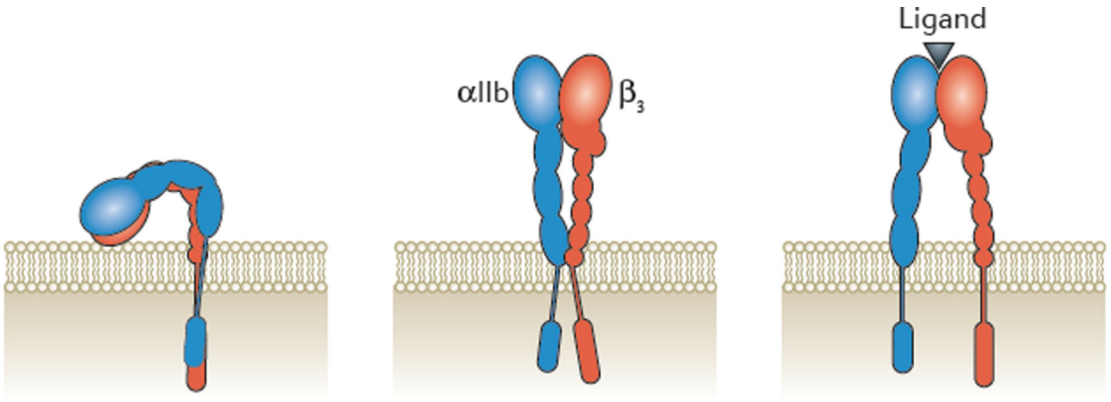


Figure 1.

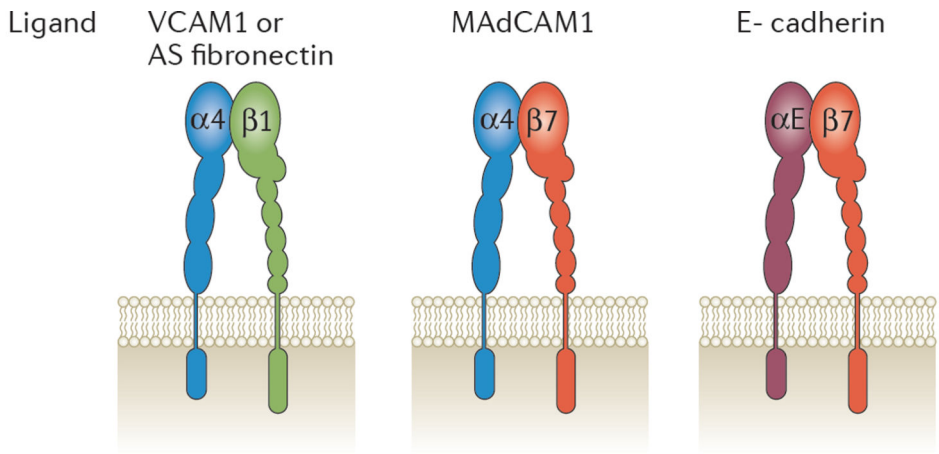
Integrin families. Integrins targeted for therapy circled in red; dotted red circle indicates past therapeutic use (for αLβ2) or unknown effects (antibodies to β7 integrins also target αEβ7, but α4β7 is believed to be the effective target). RGD-binding integrins circled in blue, I-domain containing integrin α subunits circled in green.



Affinity	Low	Intermediate	High
Conformation	Bent	Extended	Open
Outside-in signalling	No	No	Yes

Figure 2.

Inside-out activation of αIIbβ3 platelet integrin. Drawing of the bent (left), extended (middle) and extended-open (right) conformation of αIIbβ3. αIIb in blue, β3 in red. Ligand binding site indicated by black triangle in extended-open integrin. Note the movement of the transmembrane and cytoplasmic domains with integrin activation. Binding of cytoplasmic adaptor molecules (not shown here) are thought to drive the conformational changes in the ectodomains. Ligand binding affinity, conformation and outside-in signaling noted below each conformation.



Natalizumab	Yes	Yes	No	Yes
Vedolizumab	No	Yes	No	Not observed
Etrolizumab	No	Yes	Yes	Unkonwn

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Figure 3.

The three integrins α4β1, α4β7 and αEβ7 targeted by therapeutic α4 and β7 antibodies. α4 (blue) and thus α4β1 and α4β7 are targeted by natalizumab. β7 (purple) and thus α4β7 and αEβ7 are targeted by etrolizumab. Vedalizumab and AMG-181 recognize an epitope formed by both α4 and β7 and thus is monospecific. The αE subunit (orange) contains an I domain (I). Main ligands for each integrin noted above. PML: progressive multifocal leukoencephalopathy.

Table 1

Clinical trials evaluating drugs targeting αIIbβ3 in cardiovascular disease

Drug	Indication	Major findings	Major adverse effects
Abciximab (monoclonal antibody to αIIbβ3 near MIDAS site, blocks binding of fibrinogen and other RGD ligands)	In combination with heparin and aspirin: prevention of cardiac ischemic complications in patients with ACS undergoing PCI	EPIC (2,099 patients) 35% fewer events	Major bleeding (2x more common than placebo in CAPTURE, no difference in EPISTENT), thrombocytopenia by patient antibodies against murine sequences in Abciximab
		EPILOG (2,972 patients): 65% fewer events, sustained benefit at 1 year	
		CAPTURE (1,265 patients) 29% fewer events	
		EPISTENT (2,399 patients) 51% fewer events	
Eptifibatide (disulfide-linked cyclic heptapeptide, blocks binding of fibrinogen and other RGD ligands)	NSTEMI patients undergoing elective, urgent or emergency PCI	IMPACT-II (4,010 patients) 19% fewer events	Major bleeding unchanged, minor bleeding slightly increased in IMPACT-II. Vascular access site complications reduced by early sheath removal and limiting heparin dose
		PURSUIT (10,948 patients) 10% fewer events, sustained benefit at 6 months	
		ESPRIT (2,064 patients) significantly fewer events, sustained at six months and one year	
Tirofiban (small molecule, blocks binding of fibrinogen and other RGD ligands)	Unstable angina or NSTEMI patients undergoing PCI	PRISM (3,232 patients) 32% fewer events	Major bleeding similar in PRISM, PRISM-PLUS and RESTORE. Reversible thrombocytopenia 3x more common in PRISM, no difference in RESTORE
		PRISM-PLUS (1,915 patients) 28% fewer events (compared to heparin/aspirin alone)	
		RESTORE (2,212 patients) significant reduction of events at 2 and 7, but not 30 or 180 days	

ACS: acute coronary syndrome, includes unstable angina, non-ST elevation myocardial infarction (NSTEMI) and ST elevation myocardial infarction (STEMI)

PCI: percutaneous coronary intervention

Events: primary composite cardiovascular endpoint events, including death, myocardial infarction, repeat PCI, stent or bypass at 30 days, reduction compared to placebo

Table 2

Clinical trials evaluating Natalizumab targeting α4 integrins in MS and CD

Drug	Indication	Major findings	Major adverse effects
Natalizumab (monoclonal antibody to α4 ligand binding site, blocks VCAM-1 binding)	MS patients who experienced at least one clinical relapse	reduced relapses by 68% vs. placebo in 2 trials[106]	PML, a potentially fatal complication, estimated risk 2:1000 for patients treated for more than two years [102] 6% of individuals receiving Natalizumab develop efficacy-reducing antibodies [75]
	MS patients treated with Natalizumab in combination with interferon-β	Reduced relapse and disability progression more than with interferon-β alone [107]	
	Patients with relapsing MS	reduced visual loss [108] improved assessments of health-related quality of life [109, 110] MRI evidence that the formation of new lesions was prevented [111]	
Natalizumab (monoclonal antibody to α4 ligand binding site, blocks VCAM-1 and MAdCAM-1 binding)	Patients with mild to moderate, active CD (151 <CDAI <450), 30 patients	CDAI at week 2 after infusion significantly reduced[112] compared to placebo, induction of clinical remission, as defined by a CDAI <150	Significant increase in circulating B and T lymphocytes at 1, 2, and 4 weeks
	Patients with moderate to severe CD (220 <CDAI <450), 248 patients	significantly higher chance of being in remission by week 4 to 12 [113]	No difference between treatment and placebo groups
	Patients with moderate-to-severe CD (220 <CDAI <450): ENACT study. Induction and maintenance arms in patients with CD [114] 905 patients	clinical response at week 10, defined as a decrease in CDAI score of at least 70 points. Patients on continuous Natalizumab treatment had sustained clinical response through week 60	
	CD patients with objective evidence of inflammation (elevated CRP): ENCORE trial[115], 509 Patients	clinical response (reduction of CDAI by 70 points from baseline) at week 8 significantly higher than placebo. Effect maintained through week 12	
	FDA-approved as a remission-inductive, maintenance-sustaining and steroid-sparing therapy for CD. Patients must be monitored for PML in the TOUCH program		Because of the PML risk, Natalizumab has effectively been displaced by drugs targeting β7 integrins

PML: Progressive Multifocal Leukoencephalopathy, for more detail, see box 1.

CDAI: Crohn's Disease Activity Index, ranges between 0 and 600 points[116]

CRP: C-reactive protein

VCAM-1: Vascular Cell Adhesion Molecule-1

MAdCAM-1: Mucosal Addressin Cell Adhesion Molecule-1

Table 3

Clinical trials evaluating Vedolizumab targeting α4β7 in CD and UC

Drug	Indication	Major findings	Major adverse effects
MLN02 (IgG1 monoclonal antibody to a combinatorial epitope requiring α4 and β7, blocks MAdCAM-p1 binding, manufactured in mouse myeloma cell line). MLN02 was an early version of Vedolizumab.	Patients with active UC	Significant improvement by 6 weeks, sustained at 10 and 14 weeks	No deaths, no evidence of PML (more than 2,700 patients treated: zero PML events, upper level of 95% confidence interval), no increase in opportunistic infections [117, 118] Significant anti-drug antibody formation [118] one significant infusion reaction
	29 patients with moderately severe UC [119]		
	181 patients with moderate-to-severe UC (UCCS 5 to 7) phase II study [120]	Clinical remission (UCCS 0 to 2, absence of rectal bleeding) significantly higher than placebo in the 0.5mg and 2.0 mg groups	
	185 patients with moderate-to-severe CD, double-blind placebo controlled trial [121]	Significantly higher clinical remission (CDAI score < 150) in the 2.0 mg/kg group than placebo	
Vedolizumab (humanized IgG1 monoclonal antibody to a combinatorial epitope requiring α4 and β7, blocks MAdCAM-p1 binding, manufactured in Chinese hamster ovary cells)	Phase III UC trial (GEMINI 1), 374 patients [122]	Clinical response at week 6 significantly better than placebo, significantly higher remission rates than placebo, higher mucosal healing rates	no evidence of PML (more than 2,700 patients treated: zero PML events, upper level of 95% confidence interval) low anti-drug antibody formation [118] safety data not different between drug and placebo
	Active CD: phase III CD trial (GEMINI 2), 368 patients [123]	clinical remission (CDAI score of ≤150 points) and CDAI-100 response (≥100-point decrease in the CDAI score from baseline) at week 6 significantly better than placebo, maintained at week 52	
	416 patients with CD, most with previously failed anti-TNF therapy. Multicenter double-blind phase 3 study	Remission rates and rates of CDAI-100 response at week 6 and 10 in anti-TNF non-responders significantly higher than placebo [117, 124]	

UCCS: Ulcerative Colitis Clinical Score