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Integrin antagonists as inhibitors of bone resorption: implications for treatment

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Currently 'accepted' treatments for bone disease utilise drugs that inhibit osteoclastic bone resorption; these lead to a reduction in subsequent bone loss and thence, indirectly, to an increase in bone mass and fewer fractures. Three classes of compounds currently form the mainstay of therapy for osteoporosis: oestrogens (hormone-replacement therapy), 'selective oestrogen receptor modulators' and the bisphosphonates. Problems of patient compliance, real or theoretical long-term toxicological risks and the lack of bone anabolic agents of clinical utility suggest that there is a need for the development of further novel osteoclast resorption inhibitors. Recent biological and genetic findings in the area of bone cell function have led to the identification of new drug targets. These drugs include agents that (directly or indirectly): inhibit osteoclast adhesion to bone matrix; modify osteoclast differentiation; act on the proton pump and hence affect extracellullar acidification; antagonise extracellular enzymes that are involved in bone matrix protein degradation. Particular emphasis is placed in the present review on the evaluation of antagonists of αvβ3 integrin-mediated cell adhesion for use in bone disease. The wealth of new agents being developed suggests that resorption inhibition will be the best treatment for osteoporosis in the short to medium term, with the long-term aim still being toward developing anabolic drugs or cell therapeutics.

Osteoclast: Bone resorption: Osteoporosis: Integrin

Osteoporosis places a large and growing medical and financial burden on health services in developed countries (Marcus *et al*. 1996). However, it remains a clinical area where, despite recent advances in therapy and diagnosis, there are still unmet needs (Meunier, 1999). For example, new and powerful drugs have recently been introduced that reduce osteoclastic bone resorption (newer generation and more potent bisphosphonates (Gatti & Adami, 1999) and modulators of oestrogen receptor function (Dhingra, 1999)) and impact on the incidence of fractures in the elderly. The ultimate pharmaceutical goal is, however, to develop drugs that rebuild the bone mass and structure that is lost, e.g. in response to steroids or post-menopausal oestrogen deficiency. To date, no bone anabolic drugs have yet passed into general clinical practice, although derivatives of parathyroid hormone may well do in the near future (Rittmaster *et al*. 2000). It has been argued that if the new bisphosphonates are effective at reducing hip fracture, the most clinically debilitating and costly consequence of osteoporosis, then are new resorption inhibitors needed? Whilst they are efficacious, most experts consider that existing drugs are not ideal, as they are not without toxicity and have poor long-term patient compliance (Meunier, 1999). For these reasons, the pharmaceutical industry is still developing novel anti-resorptive agents. Moreover, new indications outside the osteoporosis therapeutic domain have made the development of some research targets more attractive, e.g. in rheumatoid arthritis and cancer. In the present review the range of new osteoclast targets that are being investigated in industry will be discussed, with particular emphasis on cell adhesion receptor (integrin) antagonists.

Routes towards target discovery: osteoclast inhibitory drugs

The pharmaceutical industry has taken a number of routes to discover new molecular targets for bone disease therapeutics. Some routes have been serendipitous, a good example being the discovery of osteoprotegerin (Simonet

Abbreviation: RGD, Arg-Gly-Asp tripeptide.
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et al. 1997). Here, researchers at Amgen Inc. (Thousand Oaks, CA, USA) had a discovery programme identifying novel tumour necrosis factor receptor orthologues by genomic approaches and functional screening via the use of gene overexpression in transgenic mice. Mice transgenic for the osteoprotegerin gene, a secreted form of the tumour necrosis factor receptor family, exhibit an osteopetrotic bone phenotype. Later work showed that osteoprotegerin acts as a decoy receptor and binds a membrane-associated tumour necrosis factor-related growth factor for dendritic cells, RANKL, on osteoblasts and stromal cells, blocking the interaction of RANKL with its cellular transmembrane receptor, RANK, on osteoclasts and their haemopoietic precursors, and hence inhibiting their development and function. This work had the added importance of solving a molecular puzzle that had intrigued bone biologists for many years, the mechanism by which osteoblasts regulate osteoclast differentiation and functional activation. Osteoprotegerin, via its osteoclast inhibitory actions, is being developed for use in clinical disorders of excessive bone resorption such as osteoporosis, rheumatoid arthritis and bone metastasis (Simonet *et al*. 1997; Kong *et al*. 1999; Capparelli *et al*. 2000).

Other examples of drug target identification have followed a rational analysis of bone cell molecular biology and the identification of genes involved in genetic disorders of bone in mice and human subjects. These drugs are diverse in their targets, and range from influencing osteoclast differentiation through to modification of proteolytic enzymes involved in bone matrix destruction; some selected examples are listed in Table 1.

One key step in the process of bone resorption by osteoclasts is the recognition of, and binding to, bone matrix. Much research over the last decade has implicated a member of the integrin family of cell adhesion receptors (Isacke & Horton, 2000), αvβ3 or the vitronectin receptor, in this process (Horton, 1997; Helfrich & Horton, 1999). The status of development of antagonist drugs that interfere with αvβ3 function in bone, and hence presenting a therapeutic route in osteoporosis, is reviewed.

Cell-adhesion therapeutics: the rationale

Interactions between cells and their environment (other cells and the extracellular matrix) are crucial to normal tissue development and cellular function. Moreover, accumulating data suggest that cell adhesion receptors (Isacke & Horton, 2000) play an important role in the pathogenesis of a wide variety of diseases. Understanding the mechanisms by which the various families of adhesion molecules recognise their ligands has led to the development of novel approaches to therapy. Inhibition of normal physiological processes and reversal of pathologies with drugs targeted to such interactions are beginning to be shown to be of value in clinical situations as diverse as reversal of thrombosis, transplant rejection, vascular disease and infection; to this list should be added effects on the bone loss of osteoporosis. Data has accrued over the past decade on the distribution of cell adhesion receptors in bone cells and their function (Helfrich & Horton, 1999). These studies have particularly focused on osteoclasts and, of the known types of cell adhesion receptors, members of the integrin receptor family and their ligands have received most attention; it is also relevant that therapeutic antagonists of integrins are the furthest developed by the pharmaceutical industry. Since the pivotal demonstration by Pierschbacher & Ruoslahti (1984) that cell adhesion mediated by fibronectin could be inhibited by the simple tripeptide, Arg-Gly-Asp (RGD), then a number of other peptide sequences have been shown to recapitulate integrin–ligand interactions. The recent development (Hartman & Duggan, 2000; Miller *et al*. 2000) of a number of orally-active non-peptidic integrin antagonists, particularly based on modelling of the RGD peptide motif, suggests that treatment of a range of bone diseases may be susceptible to strategies that involve the blockade of integrin function or modulation of their expression. The process of drug development for osteoporosis has been aided considerably by an analogous set of drugs that have been developed for use in thrombosis (Hartman & Duggan, 2000; Miller *et al*. 2000). Here the platelet integrin fibrinogen receptor, gpIIbIIIa/αIIbβ3, which is structurally related to the αvβ3 integrin on osteoclasts, is targeted. These drugs are

Table 1. Target identification for osteoclast inhibitory drugs

Route for discovery	Example	Reference
Genomics		
Spontaneous mouse osteopetrosis mutations (e.g. *oc/oc*)	Proton pump	Scimeca *et al.* (2000)
Deliberate gene overexpression	OPG	Simonet *et al.* (1997)
Murine gene knockout	*c-src*	Soriano *et al.* (1991)
Human osteopetrosis gene	Carbonic anhydrase II	Whyte (1993)
Human pyknodysostosis	Cathepsin K	Gelb *et al.* (1996)
Genome wide screens for genes in single gene and multi-factorial diseases or traits (mouse, man)	Osteoporosis, high bone mass genes	Johnson *et al.* (1997), Devoto *et al.* (1998)
Cell molecular analysis		Bilezikian *et al.* (1996)
Adhesion receptors	Integrins (e.g. αvβ3)	Horton & Rodan (1996), Horton (1997)
Matrix degradation	Proteases	
Extracellular acidification	Proton pump	
Cytokines or growth factors and receptors	IL-6 receptor antagonist	
Intracellular signalling molecules, transcription factors etc.	'Kinases' (e.g. c-src)	

OPG, osteoprotegerin; IL-6, interleukin 6.

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the most advanced in development and the first integrin antagonist 'drugs' have been approved for clinical use (Tcheng, 1996; Coller, 1997; Phillips & Scarborough, 1997; Theroux, 1998); they form paradigms for potential application to bone disease.

Integrin cell adhesion receptors

The term 'integrin' was first proposed in 1987 (Hynes, 1992) to describe a family of integral membrane receptors thought to 'integrate' the intracellular cytoskeleton with extracellular matrix proteins. Integrins are heterodimeric proteins consisting of non-covalently-linked α and β polypeptide subunits. 17α Chains and 8β chains have been described that form over twenty-three distinct heterodimers (for review, see Isacke & Horton, 2000). Additionally, subunits have been cloned which are mRNA splice variants of the 'original' family members, and a number of less-well-defined integrin homologues have been identified as part of genomic sequencing projects in diverse species. Biochemical, molecular and physical analyses have revealed the protein structure of the subunits. The α and β subunits are transmembrane N-glycosylated glycoproteins with large N-terminal extracellular domains, a single hydrophobic transmembrane region and generally a short cytoplasmic domain. Electron microscopy of a number of purified integrin receptors has revealed that they are extended structures of approximately 10×20 nm, with an N-terminal globular 'head', formed by the association of the two subunits, linked to the membrane by two extended 'rod' structures.

The α subunits (Isacke & Horton, 2000) are 120–180 kDa and comprise a large N-terminal extracellular domain, a short twenty to thirty amino acid transmembrane region and a C-terminus, usually consisting of a short hydrophilic sequence forming the cytoplasmic tail. All α chains contain seven homologous tandem repeat sequences, with the C-proximal three or four sequences containing putative divalent cation-binding sites. Some integrins, including the α subunit partners of the β2 integrins, contain an inserted, or 'I', domain of approximately 200 amino acids between the second and third repeats. This domain has sequence homology with several molecules, including von Willebrand factor, which can all interact with collagen. Cytoplasmic tail sequences of α subunits are poorly conserved, with the exception of the conserved amino acid motif Gly-Phe-Phe-Lys-Arg which is involved in the transmission of signals into the cell.

The β subunits (Isacke & Horton, 2000) are generally smaller than α subunits and are between 90–110 kDa, apart from the β4 chain. The N-terminal halves of all β subunits have a high cysteine content, grouped into four forty amino acid cysteine-rich regions that are internally disulfide bonded. The cytoplasmic tails are usually short (forty to fifty amino acids), although the β4 cytoplasmic domain is substantially larger and uniquely contains four fibronectin type III repeats. Functional domains are found in the cytoplasmic tail involved in ligand binding and interaction with α-actinin, which mediates linkage between the integrin receptor and the cytoskeleton.

Integrin ligand specificity

Physico-chemical analysis of integrins in conjunction with cross-linking studies with radioactively-labelled RGD peptide probes has revealed that the ligand binding site for the integrin (αvβ3) heterodimer resides in its globular head, and includes the cation-binding region of the α subunit and the N-terminal portion of the β subunit. Thus, ligand binding by integrins, and hence their specificity, is likely to depend on the particular αβ subunit combination.

In the main, integrins act as cell-surface receptors for matrix proteins. However, they can also serve as cell–cell adhesion molecules, by recognising counter receptors on other cells; for example, the β2 integrins interact with intercellular adhesion molecules in lymphocytes. Such interactions are likely to occur during osteoclast precursor differentiation in the bone marrow micro-environment (Horton & Rodan, 1996). There is also extensive literature demonstrating a role in signal transduction via integrin–ligand interactions (Clark & Brugge, 1995; Hughes & Pfaff, 1998).

Some integrins, e.g. αvβ3, can bind a number of ligands, whereas others integrins are more restricted. Conversely, several matrix proteins are recognised by a number of different integrin receptors; thus, laminin is recognised by at least seven integrin dimers. Added complexity is produced by different integrins recognising different regions of the same molecule (e.g. αvβ3 and gpIIbIIIa bind distinct sites on fibrinogen) and splice variants show differing ligand affinities.

Integrin peptide recognition motifs

The first integrin binding motif defined was the RGD sequence (Ruoslahti, 1996), identified as the minimal binding site in fibronectin that is capable of supporting cell adhesion (Pierschbacher & Ruoslahti, 1984). Several hundred RGD sequences exist in protein and DNA databases. A majority of matrix proteins isolated to date seem to contain RGD or homologous sequences, although not all are biologically active *in vivo*, and many are so only after 'denaturation'. Progressive truncation from parent matrix molecules, and adhesion inhibition and competition studies with various synthetic peptides and phage display libraries, have confirmed that many integrins recognise the RGD motif in their ligands.

αvβ3 vitronectin receptor in bone biology

αvβ3 is a member of the integrin superfamily showing the typical structural features of other integrin αβ dimers; it shares its β chain, β3, with its related integrin of platelets, gpIIbIIIa (αIIbβ3; Horton, 1997). αvβ3 protein was first purified from placenta by Pytela *et al*. (1985), and the α and β chain cDNA cloned by Suzuki *et al*. (1986) and Fitzgerald *et al*. (1987) respectively. The receptor exhibits an affinity for vitronectin (Pytela *et al*. 1985), hence its name 'vitronectin receptor', but later was found to bind to a large range of RGD sequence-containing proteins (for review, see Horton & Rodan, 1996; Horton, 1997). Binding between αvβ3 and its ligands is primarily sensitive to inhibition by

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RGD peptides, providing a rational basis for the development of antagonists based on this tripeptide structure.

In vivo, the highest levels of αvβ3 are found in the osteoclast. Lower levels of αvβ3 have been found in platelets and megakaryocytes, kidney, vascular smooth muscle, some endothelia and placenta (Horton, 1997). In pathology, tissue levels are increased; for example, tumour microvessels show increased levels of αvβ3 as do melanoma cells when they metastasise (Horton, 1997).

The role of αvβ3 in osteoclast biology was first examined by Horton and colleagues (Chambers *et al*. 1986; for review, see Horton & Rodan, 1996; Helfrich & Horton, 1999) over a decade ago, and shown to be restricted in tissue distribution in bone to osteoclasts but not osteoblasts, and to mediate cellular adhesion to a number of proteins that are found in bone matrix. A further two integrin receptors have been found in mammalian osteoclasts, α2β1 and αvβ1 (Nesbitt *et al*. 1993), although their function is less well understood; there is evidence for the involvement of α2β1 in recognition of native collagen by osteoclasts (Helfrich *et al*. 1996), and it is likely that it plays a subsidiary role to αvβ3 in the recognition of bone matrix. The exact ligand(s) that osteoclast αvβ3 recognises in bone and its role in the maintenance and function of the so-called osteoclast tight seal (Väänänen & Horton, 1995; Stenbeck & Horton, 2000) remain controversial. Its high levels in osteoclasts, the sensitivity of bone resorption to receptor blockade using synthetic RGD peptides, snake venom proteins (e.g. echistatin, a low-molecular-weight RGD-containing snake venom protein which is a potent but non-selective inhibitor of integrin function) and anti-receptor antibodies (Chambers *et al*. 1986; Sato *et al*. 1990; Horton *et al*. 1991, 1993) made this receptor a strong candidate for pharmaceutical manipulation (Horton & Rodan, 1996; Hartman & Duggan, 2000; Miller *et al*. 2000). Later, a pivotal study demonstrated that echistatin blocked the calcaemic response to parathyroid hormone in thyro–parathyroidectomised mice (Fisher *et al*. 1993). This finding put αvβ3 antagonist development clearly in the pharmaceutical mainstream for drug development for osteoporosis and other bone diseases associated with high levels of bone resorption.

Strategies for therapeutic modification of integrin function

From basic principles, there are two main strategies for therapeutically inhibiting cell adhesion molecule function (Table 2). First, a direct approach; competitive antagonists of receptor–ligand interaction can be developed, which has been the usual pharmaceutical approach, with the aim of producing orally-active, synthetic non-peptide mimetic agents. They have been identified by a variety of standard techniques of the industry, as indicated in Table 2 (Gould, 1993; Cox *et al*. 1994; Gadek & Blackburn, 1996; Samanen, 1996; Ferguson & Zaqqa, 1999; Wang *et al*. 2000). Other approaches, such as using receptor-specific antibodies, peptides, and naturally-occurring protein antagonists, together with molecular engineering, have generally been used in 'proof of principle' experiments rather than as clinical drug candidates, although there are some notable examples of protein therapeutics in the field (for examples, see Table 2). Directly-acting antagonists have entered clinical trial to modify activation-dependent platelet aggregation in thrombotic conditions via the integrin platelet fibrinogen receptor, gpIIbIIIa/αIIbβ3. Thus, groundbreaking clinical trials (EPIC, EPILOG etc.; Tcheng, 1996) have demonstrated efficacy of the humanised anti-gpIIbIIIa monoclonal antibody 7E3 (ReoPro) in various ischaemic heart conditions (Coller, 1997). Results from trials with RGD mimetics (e.g. lamifiban, tirofiban; Theroux, 1998; Ferguson & Zaqqa, 1999; Wang *et al*. 2000) and the cyclic Lys-Gly-Asp peptide, integrilin, have been less impressive (Theroux, 1998; Phillips & Scarborough, 1997). As with gpIIbIIIa-specific agents, the possibility of developing osteoclast αvβ3 (vitronectin receptor) antagonists as resorption inhibitors in bone disease was initially demonstrated *in vitro* by the use of a variety of techniques to disrupt receptor function (Horton & Rodan, 1996). Small-molecule inhibitors of αvβ3 are now at the late stage of preclinical development (Hartman & Duggan, 2000; Miller *et al*. 2000). Thus, the general principles for the use of adhesion receptor antagonists in disease have been established, and useful drugs are thus likely to be available for a wide variety of indications in the future.

The second approach is indirect, with the aim of modifying expression or intracellular function (such as signal transduction) of cell adhesion molecules, especially of integrins. Some examples of such strategies are given in Table 2. The furthest advanced of these strategies is the use of antisense oligonucleotide inhibitors of receptor protein synthesis; inhibitors of intercellular adhesion molecule 1 expression (Yacyshyn *et al*. 1998) are finding promise in the treatment of various inflammatory diseases such as those of the bowel or eye. Likewise, a number of agents to block the function of c-src, a cellular kinase that acts downstream in the signalling pathway of integrin receptors in bone cells, are being developed for treatment of osteoporosis, based on the earlier finding that deletion of *c-src* in mice led to osteopetrosis via inhibition of osteoclastic resorption (Soriano *et al*. 1991).

Selectivity for αvβ3

Early studies concentrated on specificity and potency comparisons between αvβ3 and gpIIbIIIa using linear, cyclic and modified RGD-containing peptides (Gadek & Blackburn, 1996; Hartman & Duggan, 2000; Miller *et al*. 2000). Such studies led to an early understanding of the spatial requirements for an αvβ3 mimetic, and these features were 'grafted' onto a variety of central constrained chemical scaffolds; the selection of template is usually dependent on proprietary considerations (e.g. benzodiazepine scaffolds, used by SmithKline Beecham (King of Prussia, PA, USA) in their series of gpIIbIIIa antagonists (Samanen *et al*. 1996), have been utilised for the development of αvβ3 antagonists; Hartman & Duggan, 2000; Miller *et al*. 2000).

Early peptide data, showed that echistatin, for example, although non-selective, was a potent inhibitor of αvβ3 and, moreover, blocked osteoclastic bone resorption *in vitro* (Sato *et al*. 1990) and *in vivo* (Fisher *et al*. 1993; Yamamoto *et al*. 1998). Furthermore, cyclic RGD peptides, based on the work of Kessler and colleagues

Table 2. Strategies for therapeutic modification of adhesion receptor function *in vivo*

Direct approaches
Naturally-occurring protein inhibitors and their engineered derivatives (e.g. RGD-containing snake venoms and proteins from ticks and leeches etc.)*
Blocking antibodies, and their engineered derivatives, to adhesion molecules†
RGD peptides and their chemical derivatives (e.g. designed to improve specificity and stability)‡
Oligosaccharide analogues (selectin adhesion receptor inhibition)
Receptor–Ig chimeras
Non-peptidic mimetics§, produced via different compound selection strategies‖
Indirect approaches
Altered receptor synthesis via use of antisense oligonucleotides¶
Inhibition of adhesion receptor expression via regulatory cytokines and their receptors (e.g. in endothelium)
Modification of integrin receptor function via adhesion molecule (integrin)-associated proteins
Modulation of receptor affinity for ligands (e.g. via integrin activation), and hence adhesion
Modification of downstream receptor-associated signalling (e.g. c-src and other kinases, adhesion-associated apoptosis genes etc.)

RGD, Arg-Gly-Asp tripeptide; Ig, immunoglobulin.

* Echistatin has been used as a proof of concept inhibitor of αvβ3 in bone disease studies (Fisher *et al.* 1993); Barbourin snake venom protein contains Lys-Gly-Asp (KGD) instead of RGD and is the basis of selective inhibitory analogues for platelet gpIIbIIIa (Phillips & Scarborough, 1997).

† Antibodies to gpIIbIIIa (i.e. 7E3, ReoPro; Centocor Inc., Malvern, PA, USA) formed the first integrin of the cell adhesion receptor inhibitors licensed for clinical use in the various vascular and thrombotic conditions (Tcheng, 1996; Coller, 1997). A humanised αvβ3 antibody (clone LM609) is currently in clinical trial for cancer acting via induction of apoptosis in tumour vessels.

‡ Integrilin (Cor Therapeutics Inc., South San Francisco, USA), a cyclic KGD-containing peptide gpIIbIIIa inhibitor is in clinical trial (Coller, 1997; Phillips & Scarborough, 1997), as are RGD-derived cyclic peptides with selectivity for αvβ3 (cyclic RGDfVA; E. Merck, Darmstadt, Germany; Haubner *et al.* 1996).

§ A number of companies have intravenous and orally-active non-peptidic gpIIbIIIa antagonists in clinical trial for platelet-related disorders (Coller, 1997; Phillips & Scarborough, 1997; Theroux, 1998). Analogous mimetics are in late preclinical development for inhibition of αvβ3 (Horton & Rodan, 1996; Gadek & Blackburn, 1996; Hartman & Duggan 2000; Miller *et al.* 2000) in bone disease and cancer etc., and to modify β2 integrin–intracellular adhesion molecule and –α4β1 interactions in inflammatory disorders, transplantation etc. (Gadek & Blackburn, 1996; Lin & Castro, 1998).

‖ Structure–function, combinatorial chemistry, phage display, compound or natural product library screening etc. (Lazarus & McDowell, 1993; Pasqualini *et al.* 1995; Corbett *et al.* 1997; Kunicki *et al.* 1997; Hoekstra & Poulter, 1998).

¶ Antisense therapeutics directed against intracellular adhesion molecule 1 (Isis Pharmaceuticals Inc., Carlsbad, CA, USA) in inflammatory bowel disease are showing promise in clinical trials (Yacyshyn *et al.* 1998).

and developed by E. Merck (Darmstadt, Germany), were shown to display selectivity and potency for αvβ3 (Pfaff *et al*. 1994; Haubner *et al*. 1996). Similar results were found by a number of other groups using cyclic and modified RGD peptides, and many of these peptides were shown to block osteoclast function *in vitro* (Horton *et al*. 1993). More recent work by the pharmaceutical industry has concentrated on the development of non-peptide mimetics of αvβ3 function (Hartman & Duggan, 2000; Miller *et al*. 2000).

Current drug development status for use in bone disease

The action in bone models of several early candidate mimetic αvβ3 antagonists has been reported by a number of companies, and their evolution has been reviewed recently (Hartman & Duggan, 2000; Miller *et al*. 2000); as yet, these are not drugs, but agents used for 'proof of concept' and pharmaceutical experiments. Compounds based on a variety of scaffolds from Merck & Co. (L-748,415; Rodan *et al*. 1996), GD Searle (St Louis, MO, USA; SC-56631; Engleman *et al*. 1997), SmithKline Beecham (SB 265123 and other compounds; Miller *et al*. 1999; Lark *et al*. 1999) and HMR (Paris, France)/Genentech Inc. (South San Franciso, CA, USA; Peyman *et al*. 2000), which have all shown varying efficacy and specificity for αvβ3 in the number of *in vitro* screening assays, have inhibitory effects on the calcaemic response in thyro–parathyroidectomised rodents and bone-sparing responses in ovariectomy models (Hartman & Duggan, 2000; Miller *et al*. 2000). Drug candidates with optimised pharmacokinetics and dynamics are about to enter clinical trials for bone disease, and for other indications where αvβ3 is involved in disease pathogenesis.

Expert opinion

Preclinical studies, taken with positive findings in 'proof of concept' studies using small-molecule mimetics in a number of *in vivo* models of bone metabolism, underline αvβ3 antagonists as promising candidates for a new class of bone disease therapeutics. Whilst many of these agents are orally active, there are still several outstanding issues, e.g. pharmacokinetic and pharmaceutical, that require further optimisation. Moreover, the expression, albeit at lower levels, of αvβ3 in other tissues suggest that other end points may produce unwanted side effects; for example, will they interfere with wound healing? Specificity issues may also be important in defining the toxicological profile of αvβ3 antagonists; thus, will these drugs interfere with the function of the related integrin, αvβ6, in epithelia, and hence produce significant respiratory tract or gut side effects?

Finally, although αvβ3 antagonists have been developed for use in bone diseases, other clinical targets also show promise. For example, diseases such as rheumatoid arthritis, angiogenesis in eye diseases and cancer, vascular restenosis following coronary angioplasty, and targeting of invasive melanoma and other tumours are all being investigated for possible new applications of αvβ3 antagonist drugs.

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