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Alpha4 revisited — this approach to the treatment of inflammatory disorders bounces back!

Alpha4-integrin antagonism — an effective approach for the treatment of inflammatory diseases?

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Inhibition of leucocyte trafficking by antagonism of the alpha4 (α4)-integrin has now been validated as a therapeutic approach for the treatment of inflammatory diseases such as multiple sclerosis (MS) and inflammatory bowel disease (IBD). This validation has been overshadowed by three incidences of progressive multifocal leucoencaphalopathy (PML) in patients receiving natalizumab (Tysabri®), a therapeutic monoclonal IgG antibody directed against α4-integrins. This led to the initial removal of natalizumab from the market. Following a safety review, it was reintroduced for the treatment of relapsing-remitting MS patients (with restrictions). This has led to a refocus on α4-integrins as a therapeutic target across the pharmaceutical industry. Recent advances in small molecule development are worth reviewing. New understanding of pharmacokinetics and selectivity will potentially contribute to the development of α4 antagonist with greater clinical efficacy and safety.

Alpha4-integrins

Integrins are integral membrane proteins, which play a role in cell adhesion and signal transduction. Integrins are heterodimers consisting of α and β subunits [1]. Association of specific α and β subunits governs their ligand specificity. The alpha4 (α4)-integrin family comprising α4β1 (very late antigen-4, VLA-4) [2] and α4β7 [3] has been shown to be important cell adhesion proteins involved in directing leucocyte trafficking to sites of inflammation (Figure 1).

Leucocytes expressing either α4β1 or α4β7 are captured from the circulation by vascular cell adhesion molecule-1 (VCAM-1) or mucosal addressin cell adhesion molecule-1 (MAdCAM-1) expressed on the endothelial cell layer. Changes in affinity of α4β1 or α4β7 enable firm adhesion followed by transmigration across the endothelial cell layer towards the site of inflammation

α4β1-Integrins are expressed at moderate-to-high levels on almost all lymphocytes, monocytes and eosinophils. α4β1 is a receptor for the immunoglobulin adhesion ligand VCAM-1 and for fibronectin. Importantly, VCAM-1 is up-regulated by inflammatory cytokines on the endothelium during diseases such as multiple sclerosis (MS). The interaction of α4β1 with VCAM-1 leads to the recruitment of leucocytes to sites of inflammation *in vivo*. In contrast, α4β7 is expressed on a more restricted set of leucocytes including activated macrophage, subsets of lymphocytes, NK cells, mast cells and eosinophils. α4β7 is a receptor for the immunoglobulin receptor ligand MAdCAM-1 [3], which is specifically expressed on the intestinal endothelium and helps direct lymphocyte traffic into the Peyers patch and the intestinal lamina propria. MAdCAM-1 is up-regulated in the gut during inflammation and is believed to play a role in diseases such as ulcerative colitis (UC) and Crohn's disease (CD).

On the basis of results obtained in animal models, supplemented where possible by clinical data, it was concluded that antagonism of α4β1 holds promise as an effective therapeutic approach to asthma, rheumatoid arthritis (RA) and multiple sclerosis (MS) [4], whereas blocking α4β7 may open up novel treatments in the management of inflammatory bowel disease (IBD) [4]. The only α4 antagonist available on the market is natalizumab, a therapeutic monoclonal antibody directed against the α4 chain of both α4β1- and α4β7-integrins.

Elan and Biogen have jointly developed Natalizumab (Tysabri®, originally known as Antegren®) primarily for treatment of MS, but

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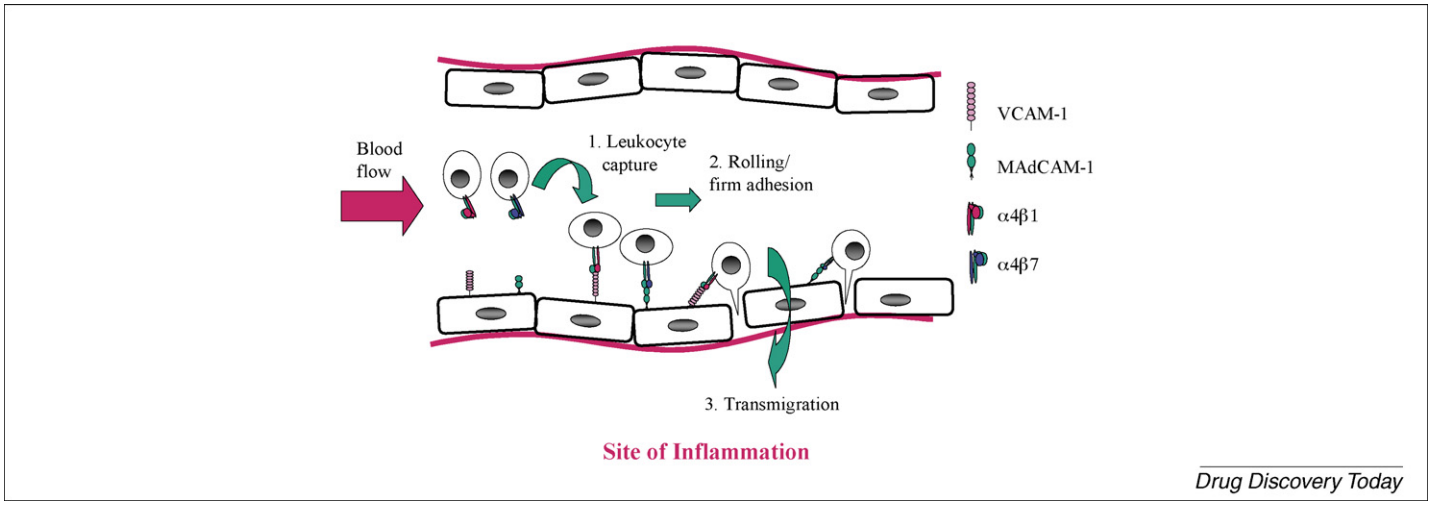

FIGURE 1

Leucocyte trafficking mediated by α4-integrins.

the drug has also undergone clinical trials for the treatment of CD [5] and rheumatoid arthritis (ClinicalTrials.gov identifier: NCT00083759). The key role of α4-integrins in the development of multiple sclerosis was first demonstrated in murine models (Figure 2) of experimental autoimmune encephalomyelitis (EAE). Neutralising α4 with the use of a murine antibody effectively prevented the accumulation of leucocytes in the central nervous system (inflammatory lesions) and the development of EAE [6]. The antibody used in this study was subsequently humanised to yield the therapeutic IgG-neutralising α4 antibody natalizumab, which also showed efficacy in the guinea pig EAE model [7,8].

The launch of natalizumab [8] in November 2004 was on the back of one 6-month serial MRI study [9] and one-year results of two two-year Phase III clinical trials, the AFFIRM monotherapy trial [10] and the SENTINEL trial [11] in which patients were co-dosed with natalizumab and intramuscular interferon β-1a (Avonex®). Using serial MRI scanning in Phase II, natalizumab showed marked reductions in both the formation of new lesions and the enlargement of existing lesions. In the AFFIRM study, natalizumab showed a reduced rate of clinical relapses (68%) relative to placebo at one year. This led to 77% of patients remaining relapse free in the first year in the patient cohort receiving natalizumab, compared to 56% in the patient group receiving the placebo. In the SENTINEL trial, it was hoped that co-administering natalizumab with Avonex® would yield additional benefits for those MS patients who, despite Avonex® therapy, had had at least one

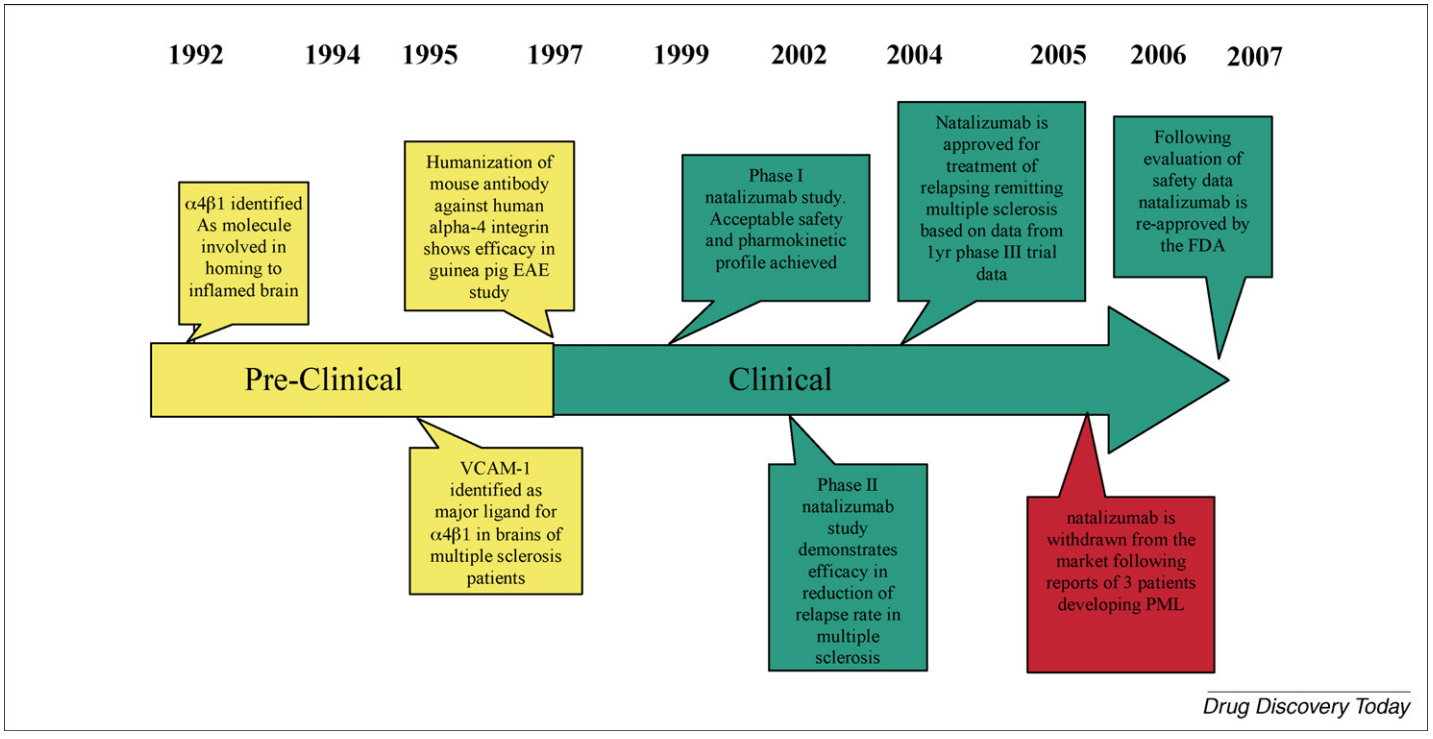

FIGURE 2

Key milestones for the development and launch of natalizumab.

relapse during the past 12 months. Co-administration of natalizumab with Avonex® led to a 54% reduction in relapses versus administration of Avonex® alone. These impressive data one year into these two trials led to a priority review by the FDA and subsequent accelerated approval of natalizumab. Natalizumab was launched in November 2004 for the treatment of relapsing forms of MS. Full two-year data from the AFFIRM trial have subsequently shown a 42% reduction in the relative risk of sustained disability progression compared to the placebo. The reduction in the number of clinical relapses was maintained. Also, two-year data from the SENTINEL trial demonstrated a reduction in the relative risk of sustained disability progression (24%) and maintained the reduction in the relapse rate too.

In parallel with the MS trials, Biogen and Elan also actively investigated the potential of natalizumab for the treatment of CD. Three Phase III trials were performed, starting with the ENACT-1/2 trials [5] with 905 CD patients with moderate-to-severe disease. ENACT-1 failed to reach its primary efficacy endpoint, with response and remission rates at week 10 being 56% and 37% for the natalizumab-treated group versus 49% and 30% for the placebo group. In ENACT-2, 339 patients who showed a response to natalizumab in ENACT-1 were reassigned to receive 300 mg of natalizumab or placebo every four weeks up to week 56. Continuing natalizumab in the second trial resulted in higher rates of sustained response (61% vs 28%) and remission (44% vs 26%) through week 36 than did switching to placebo. In light of this uncertainty, a further Phase III trial, called ENCORE, was initiated in the United States with recently released results [12] indicating that natalizumab induced response and remission by week 8 that was sustained through week 12, thus meeting its primary efficacy endpoint for clinical response.

Enthusiasm for α4 antagonism was dampened in February 2005 when Biogen and Elan voluntarily withdrew natalizumab from the market and suspended all its ongoing clinical trials. This was a consequence of two cases of progressive multifocal encephalopathy (PML), which was followed later by a third case [13–15]. PML is a rapidly progressive demyelinating disease caused by opportunistic infection of oligodendrocytes and astrocytes in the CNS by the JC virus [16]. At this point, the FDA took the precautionary measure of stopping all clinical trials involving α4 antagonists. Though mechanistically plausible, a direct connection between natalizumab treatment and the development of PML is still a subject of debate. Recently, a study was carried out on all patients who had undergone clinical trials with natalizumab. A total of 3116 patients (out of the 3417 who had received natalizumab treatment) with a mean duration of 18 months of natalizumab exposure were assessed. This evaluation showed that three previously identified cases of PML could be confirmed by a follow-up MRI analysis. The study estimated a risk of approximately 1:1000 for developing PML following natalizumab treatment [17]. Following this review, Biogen and Elan put forward a supplemental biologics license application for the reintroduction of natalizumab as a monotherapy treatment for relapsing forms of MS that was approved by the FDA in June 2006. This successful reintroduction did come with restrictions that included a revised label and risk management plan. Later in 2006, the European Medicines Agency and the Canadian regulatory agency approved natalizumab with similar restrictions. The successful regulatory (re)submission of natalizumab has given companies the green light to restart their α4 clinical trials and has also led to a recent resurgence of work in the field of small molecule α4 antagonists.

Alpha4-integrin antagonists in development

Even with an α4 antibody therapy (natalizumab) available on the market, small molecule α4 antagonists are still a very attractive prospect. The cost of goods of small molecules tends to be much lower than that of expensive antibody treatments. Small molecule dosing can be managed by the patients in their home environment and does not require a hospital or surgery visit every month. As such, small molecule antagonist research in the α4-integrin area has now been going for more than 10 years and has seen many hundreds of patents published. The types of antagonists that have been discovered can be categorised into two main classes, with both classes yielding compounds that have progressed to development. The first class can be traced back to an early publication from Tanabe [18] of the cyclic peptide **1** (Figure 3), which has been shown to be a relatively potent antagonist of α4β1 as well as of α5β1. Early discoveries from sequence work performed by Genentech [19] advanced the understanding of SAR by establishing that neither the arginine nor the aspartic acid moiety of **1** is key for activity. Further studies established that the inclusion of a phenylalanine in the cyclic peptide was beneficial. A number of other companies including Roche [20,21], AstraZenecca [22] and Biogen also took great leaps forward in simplifying the structure of **1**. They all observed that a key structural element of all α4 small molecule antagonists described to date is a carboxylic acid, which is believed to form a key binding interaction with a cation in the α4-integrin subunit [23]. The anionic nature imposed by this functional group, coupled with the relatively high molecular weight and polar surface areas often required to deliver potent antagonist, leads to many compounds having reduced gastrointestinal absorption potential. To circumvent this, medicinal chemists have pragmatically resorted to using prodrug esters of the acids to ensure delivery to the systemic circulation and enable convenient oral dosing regimes. The pharmacokinetic profile of all α4 antagonists is further complicated by a general lack of metabolism, with elimination predominantly *via* hepatic uptake and biliary excretion. Although biliary excretion *per se* is not an issue for a compound under development, high rates of elimination do lead to a reduction in exposure. Because of the lack of appropriate *in vitro* systems to model such processes, rational drug design for reducing clearance has proved extremely difficult, with programmes taking an empirical approach and relying on Phase I human data. Recently, hepatocyte sandwich cultures [24–26] have been constructed, fully characterised and used to predict biliary elimination of a limited set of non-α4 antagonists. However, as yet, they have not impacted on α4 antagonists programmes.

Urea-based alpha4-integrin antagonists

These key discoveries led to improved potency and binding efficiency of α4-integrin antagonists, with reasonable pharmacokinetics. The first clinical candidate was Bio-1211 (**2**), developed by Biogen [27]. Bio-1211 is a potent and selective inhibitor (4 nM) of α4β1/α4β7-mediated binding. After a 0.1 mg/kg nebulised dose in sheep sensitised to *Ascaris suum* asthma model, Bio-1211 inhibited the early-phase and almost eliminated the late-phase increase in

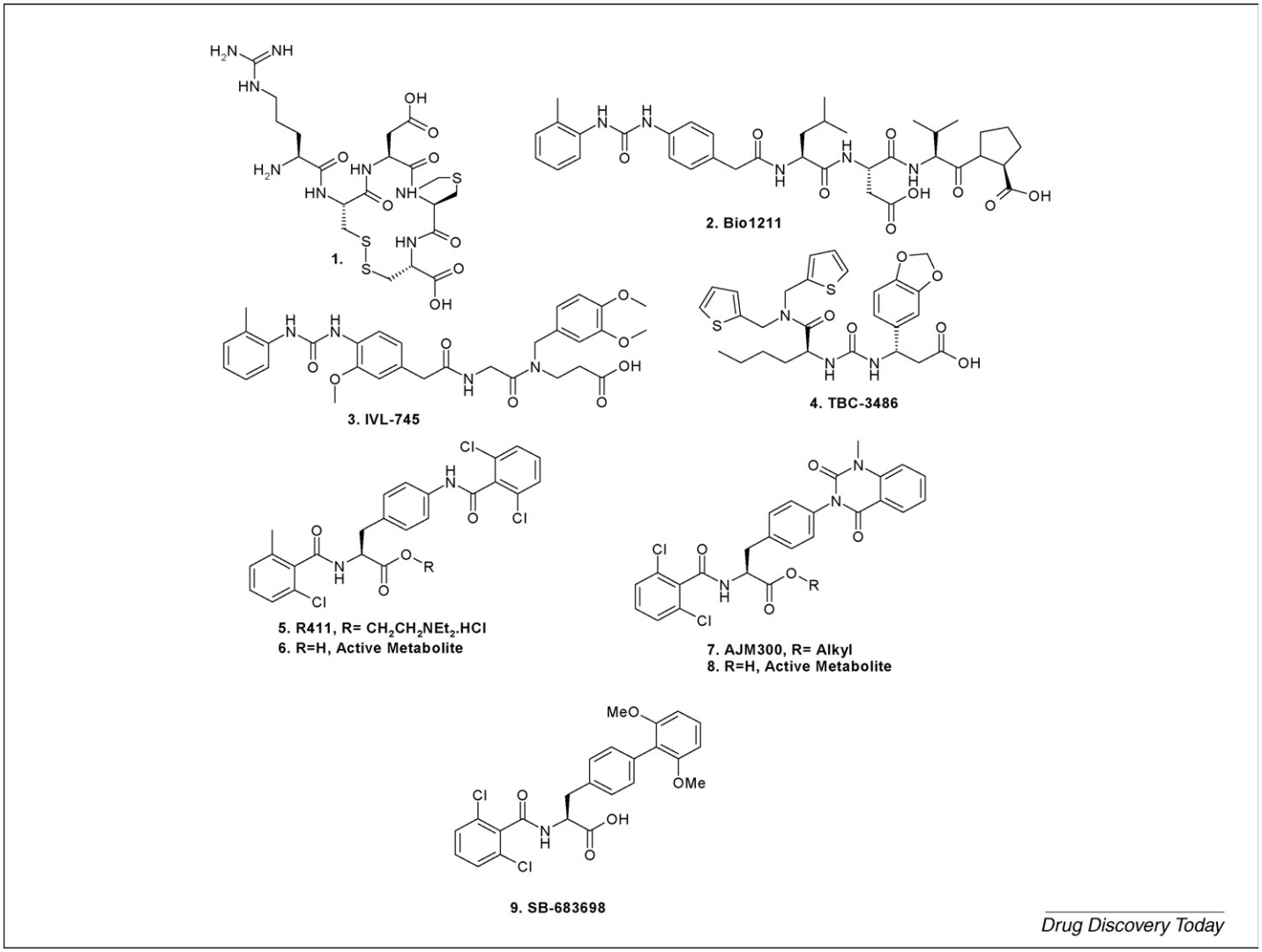

FIGURE 3

α4 Antagonists in development.

bronchial resistance 24 hours after challenge. These results led to Bio-1211 being licensed to Merck & Co., who progressed the compound as a nebulised formulation into the clinic for evaluation as a treatment for asthma. The compound was dropped due to disappointing efficacy results in Phase II trials, probably as a result of poor exposure. Other companies have pursued α4 antagonists within the same structural class as Bio-1211 [28], mainly attempting to increase efficacy, but also trying to improve exposure, and reducing clearance by reducing the peptidic nature of the candidates. IVL-745 (**3**) appeared to be the most advanced of these, which Aventis took into the clinic as an inhaled anti-asthmatic agent. IVL-745 (**3**) was a potent selective inhibitor of VCAM binding to Ramos cells, which showed efficacy in a dose-dependent manner when administered in an allergic Brown–Norway rat model [29], where it prevented lung histopathological changes and eosinophil accumulation. Unfortunately, when patients with mild asthma were dosed in a Phase II study, IVL-745 showed no effect on the primary biomarkers of the studies and only a slight reduction in sputum eosinophils [30,31]. Although Aventis has not published the discontinuation of IVL-745, its development no longer appears in the company's pipeline (http://www.aventis.com/). Interestingly, both Biogen and Aventis (and also subsequently GSK [32]) have all compounds from both the urea-based and phenylalanine classes that had failed in the Phase II clinical trials because of poor efficacy in asthma patients when dosed in an inhaled regime. This is probably because systemic exposure is actually required for efficacy. In animal models, the high doses used probably ensured systemic exposure and led to the observed efficacy. Once this dose level was dropped for the human trials, no systemic exposure and hence no efficacy was observed. The fact that this localised route of delivery for these compounds was attempted highlights the challenges of attaining small molecule α4 antagonists with acceptable oral bioavailability ($F > 20\%$, oral bioavailability (F) = the percentage of the dose that reaches the systemic circulation after oral administration) and exposure.

Texas Biotechnology (now called Encysive Pharmaceuticals) has reported a series of potent urea-based antagonists, of which TBC-3486 (**4**) appears to be one of the most advanced. TBC-3486 has shown efficacy in both the mouse lung inflammation and the adjuvant arthritis models at doses as low as 3 mg/kg i.p. [33–35]. Texas Biotechnology entered into a developmental collaboration with Schering Plough in 2000, which led to the discovery of

TBC-4746, which entered Phase I clinical trials in March 2005. TBC-4746 is believed to belong to the same structural class as TBC-3486, and as such, it is suspected that to obtain oral absorption, TBC-4746 has to be dosed as a prodrug. If Phase I data are positive, Schering Plough intends to progress TBC-4746 into Phase II trails as a treatment for asthma and MS. Daiichi and Pharmacopeia [36] have also worked on a similar structural class and have recently disclosed that DW-908e (structure unknown) has entered Phase I clinical trials.

Phenylalanine-based alpha4-integrin antagonists

Phenylalanine-based structures represent the second major class of small molecule α4 antagonists. One of the most advanced compounds in development is the prodrug ester R-411 (**5**) (US adopted name Valetegrast Hydrochloride) from Hoffman La Roche [37]. R-411 is currently in Phase II clinical trials for asthma. The Phase I data published [38] recently have shown a dose-proportional exposure to the active 'acid' metabolite (**6**), with no accumulation of drug occurring after multiple administrations of the prodrug. The absolute bioavailability under fasting conditions was 27%, with a half-life of seven to nine hours, indicating the potential for a once-a-day dosing regime. No serious adverse events were reported. Early Phase II data [39] suggest that a clinical reduction in asthma was obtained, ranging between 25 and 50%; however, no dose–response relationship was observed. Additional studies are still ongoing to determine the full potential of R-411 in asthma, although a recent company R&D update suggests that R-411 is now being developed exclusively for MS. AJM-300 (**7**) is a small molecule currently being developed by the Japanese company Ajinomoto. The company reports that AJM-300 has shown dose-dependent efficacy at 1, 3 and 10 mg/kg in a DNBS-induced colitis model and has passed successfully through Phase I clinical trials. No major adverse events were observed and as of November 2005 when AJM-300 was in Phase II clinical trials for treatment of IBD (http://www.ajinomoto.com/ar/I_r/pdf/presentation/FY2004data.pdf). Although the structure of AJM-300 has not been disclosed, patent analysis [40] suggests that it probably has the structure shown in Figure 3 (**7**) and, like many other α4 antagonists, it is dosed as an ester prodrug to circumvent poor oral absorption. GSK (http://www.gsk.com/financial/pp_pipeline_standard.html) and Tanabe (http://www.tanabe.co.jp/english/rd/pipeline.html) are co-developing a mixed α4 antagonist [41] SB-683699 for the treatment of MS, IBD and CD. The structure of SB-683699 has not been disclosed; however, it is believed to belong to the same structural class as SB-683698 (**9**), which entered Phase I clinical trials in 1999 but was not progressed to Phase II clinical trials, being superseded by SB-683699. Unlike many other α4 antagonists, SB-683699 is dosed as the free acid, and Phase I data have indicated that the compound may achieve efficacy on surrogate parameters comparable to natalizumab when dosed at relatively high doses (800 and 1200 mg). In November 2005, SB-683699 was progressed to Phase II clinical trial for treatment of both MS and CD. Finally, CDP-323 is a small molecule that is currently being developed by UCB (formally Celltech). CDP-323 is a phenylalanine enamide mixed α4 antagonist [42] used for the treatment of inflammatory and autoimmune disorders. Pre-clinical data for CDP-323 showed good oral bioavailability when the active metabolite was dosed as an ester prodrug across a number of species (>25%) [43]. As expected, excretion was *via* hepatic uptake and biliary elimination, although this was slow enough to enable achievement of good exposure. Efficacy in the chronic EAE mouse model has recently been disclosed, with CDP-323 significantly reducing clinical score when dosed at 100 mg/kg subcutaneously once a day, in both a prophylactic and a therapeutic setting. CDP-323 showed a clean pre-clinical toxicology profile and was progressed to Phase I clinical trials in 2003. The Phase I clinical trial completed in March 2004 showed good plasma exposure and potent and prolonged inhibition of leucocyte VCAM-1 binding. In October 2006, UCB announced a co-development and marketing agreement with Biogen Idec for CDP-323, with the compound being progressed to Phase II clinical studies for the treatment of MS.

α4β7 Selective antagonists in development

A potential safety advantage could be obtained by specifically targeting trafficking events mediated by α4β7 binding to MAdCAM-1 within the gut over α4β1 binding to VCAM-1 expressed on the endothelium in the CNS. It is proposed that inhibition of α4β1 may impose a unique risk factor for PML by diminishing viral immunosurveillance within the CNS. As a consequence, development of selective α4β7 antagonists over mixed antagonists could provide an effective and safer treatment for IBD. MLN-02 (Table 1) is a humanised antibody to the α4β7-integrin developed by Millenium Pharmaceuticals. A Phase II clinical trial with MLN-02 has shown efficacy in targeting α4β7 for treating ulcerative colitis.

TABLE 1

Stage of development of different α4-integrin antagonists

Company	Name	Action	Disease	Clinical trial status
Biogen/Elan	Natalizumab (Tysabri®)	α4 Antibody	MS	Marketed
Biogen/Elan	Natalizumab	α4 Antibody	IBD	Phase III
Millenium Pharmaceuticals	MLN-02	α4β7 Antibody	IBD	Phase II
Aventis	IVL-745	α4β1/α4β7 Antagonist	Asthma	Phase II
Roche	R-411	α4β1/α4β7 Antagonist	Asthma	Phase II
Ajinomoto	AJM-300	α4β1/α4β7 Antagonist	IBD	Phase II
GSK	SB-683699	α4β1/α4β7 Antagonist	IBD/MS	Phase II
UCB/Biogen	CDP-323	α4β1/α4β7 Antagonist	MS	Phase II
Daiichi-Sankyo/Pharmacopeia	DW-908e	α4β1/α4β7 Antagonist	Asthma	Phase I
Encysive/Schering Plough	TBC-4746	α4β1/α4β7 Antagonist	Asthma/MS	Phase I

Natalizumab – rise, fall and rise again!

MLN-02 was infused twice, 28 days apart. Clinical remission rates at week 6 were 33%, 32% and 14% for the group receiving 0.5 mg/kg MLN-02, the group receiving 2 mg/kg MLN-02 and the placebo group, respectively. Endoscopic improvement, endoscopic remission and histopathalogical improvement were found in all the treatment groups [44]. The lymphocytosis that occurs after treatment with natalizumab was not observed under treatment with MLN-02. Despite significant efficacy demonstrated with this antibody, it was found that neutralising antibodies developed in 44% of patients in this short-term trial. Clinical remission in patients with higher antibody titres was similar to that in the placebo group. Though it would be premature to conclude on the clinical relevance of neutralising anti-MLN-02 antibodies, this finding underlines the distinct advantage of small molecule antagonists in that they do not elicit such a response.

Compounds in research

Analysis of recent patents would suggest that many companies are still working in the area of small molecule α4 antagonist research. Of particular note are Ajinomoto [45] patents where inclusion of substituents in the phenyl ring of AJM-300 (**7**), as in **10** (Figure 4), leads to a dramatic reduction in clearance in rats (35–3.5 ml/min/kg). Although this may not translate to humans, it is still a reasonable assumption that this advance is a move in the right direction for achieving α4 antagonists that have increased exposure and hence improved duration of pharmacological action. Unfortunately, it would appear that these compounds still have to be dosed as ester prodrugs to achieve acceptable oral systemic exposure. Meanwhile, it seems that Tanabe may have achieved similar results, as it claims that the inclusion of an alkoxy substituent (as shown in **11**) into the structural motif **9** [46] improves the bioavailability by reducing the hepatic clearance.

As methods evolve, which enable clearance to be modulated, coupled with the opportunity to move away from using prodrug esters to achieve oral absorption, α4 antagonist research will take another leap forward. One can envisage that this next generation of α4 antagonists could potentially offer more convenient dosing regimes, better exposure, greater efficacy and reduced toxicology issues. On a different note, Johnson & Johnson (J&J) has recently reported small molecule phenylalanine-based antagonists selective for α4β7 interimn *in vitro* [47–49]. The company claims a series of analogues exemplified by **12** having nanomolar cellular activity directed against α4β7 with >100-fold selectivity for α4β7 over α4β1. If this selectivity truly does translate into a selective pharmacological action *in vivo*, this will represent a significant step forward in the field. Selective small molecule α4β7 antagonists could yield a valuable treatment for inflammatory diseases while potentially reducing the risk of PML.

Conclusions

Research into cell adhesion molecules such as α4β1 and α4β7 has now spanned over 10 years. The first therapy available on the market was an α4 antibody, natalizumab, which achieved excellent clinical efficacy and provided clinical validation of the α4 pathway for a number of inflammatory disorders, particularly MS. Unfortunately, the benefits of α4 antagonists were questioned when the potential link between α4 antagonism and PML was noted. After a thorough analysis of the clinical data and the assessment of risk versus benefit, the FDA approved reintroduction of natalizumab for relapsing MS (with restrictions). This positive news has also led to a reassessment of the benefits of the current α4-integrin clinical trials that are on hold, leading to these trials being reinitiated with new safety protocols to limit the risk of PML. This represents a clear message from the regulatory bodies that α4 antagonism as a clinical approach still is a viable way forward and is very much back on the agenda. Many in the field, including the authors, believe small molecule α4 antagonists will prove to be a valuable and effective treatment for some inflammatory disorders. The reduced cost of goods and more attractive dosing regimes of small molecule α4 antagonists will enable clinicians to offer

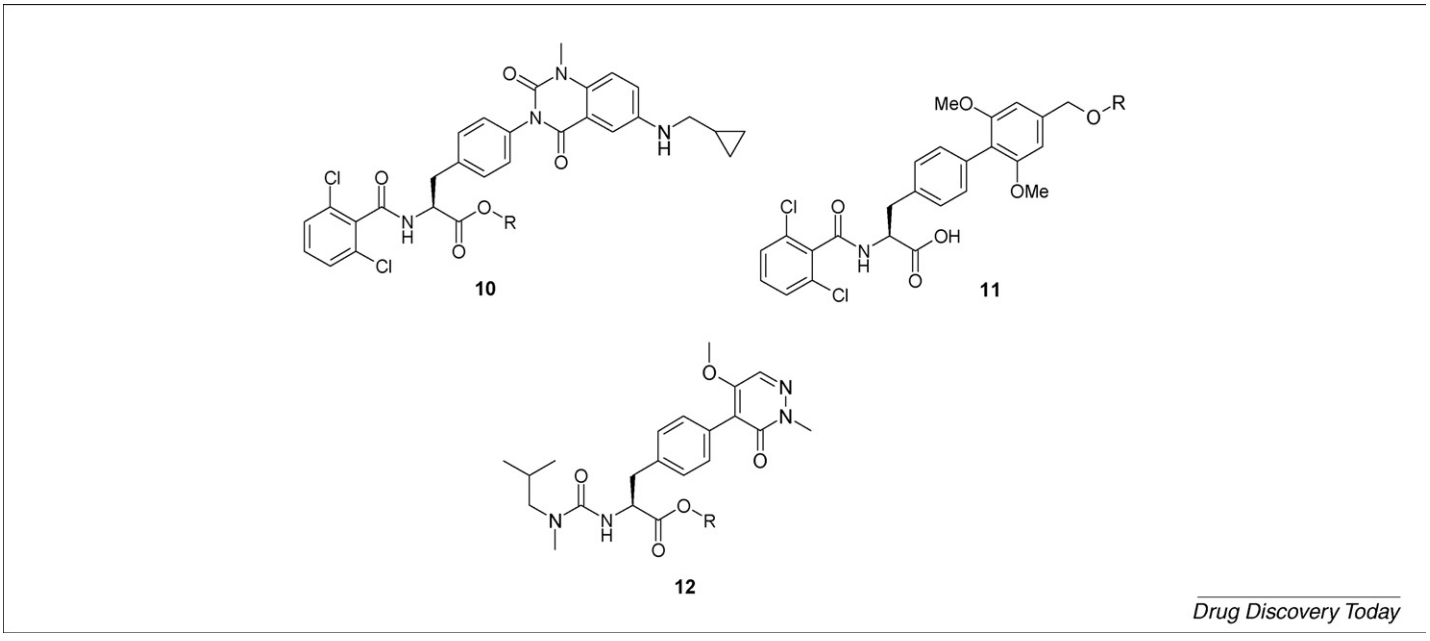

FIGURE 4

α4 Antagonists showing significant advances in research.

greater choice to their patients. It is also the authors' belief that recent advances in our understanding of hepatic uptake, biliary excretion and selective antagonism will contribute to a next generation of α4 antagonist with greater clinical efficacy and safety.

PML and natalizumab

Progressive multifocal leucoencephalopathy (PML) is a rapidly progressive demyelinating disease caused by opportunistic infection of oligodendrocytes and astrocytes in the CNS by the JC virus and was first described in 1958. In 1971, the viral nature of the disease was noted and its link to the JC virus established. Prior to AIDS pandemic, PML was incredibly rare; however, it has subsequently become more prevalent and is now a disease associated with severe immune deficiency. During the ongoing clinical trials of natalizumab, two cases of progressive multifocal encephalopathy (PML) were noted, followed later by a third case. At this point, the FDA took the precautionary measure of stopping all clinical trials involving α4 antagonists. Though mechanistically plausible, a direct connection between natalizumab treatment and the development of PML is still a subject of debate. Understanding of the potential molecular mechanisms associated with inhibition of α4β1 and reactivation of JCV still requires more basic research. However, the overall safety risk associated with this treatment has been assessed. Recently, a study was carried out on all patients who had undergone clinical trials with natalizumab. A total of 3116 patients (out of the 3417 who had received natalizumab treatment), with a mean duration of 18 months of natalizumab exposure, were assessed. This evaluation showed that three previously identified cases of PML could be confirmed by a follow-up MRI analysis. The study estimated a small risk of approximately 1:1000 for developing PML following natalizumab treatment. Further research in the area of PML will elucidate the overall impact of α4 antagonism on the occurrence of this disease. (For a more detailed discussion around PML see [50].)

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