

Executive Summary

Proteases are enzymes that exert their effects by the cleavage (catalytic proteolysis) of a peptide bond in specific substrates. Proteases constitute one of the largest (probably the largest) class of potential drug targets that can be exploited by the pharmaceutical industry. There are 561 human gene products identified that incorporate protease sequences, most of which are functionally active, and a number of mutated proteases have been identified, leading to the suggestion that there are at least 647 human proteases that have the potential to be targets for therapeutic intervention. There are many more proteases to be found in non-mammalian organisms, such as viruses and parasites, which are also potential drug targets. Inhibition of protease activity thus provides the opportunity to modulate physiological functions, either by reducing the formation of undesirable peptide mediators or by enhancing the beneficial effects of peptides by preventing their catabolism.

The term *human degradome* has been coined to describe the proteases that are found in the human genome, and the encoded proteases have been classified into five classes based upon the specific nature of the catalytic site. These classes have been subdivided into subfamilies (or clans) based upon structural characteristics. Non-mammalian proteases fall into the same basic classes but often show limited sequence homology to their human orthologs. The five classes of proteases are aspartic proteases, cysteine proteases, serine proteases, threonine proteases, and metalloproteases, where the name denotes a key feature of the catalytic site, either a nucleophilic amino acid or a metal cation.

The 21 human aspartic proteases include a number of significant drug targets, and HIV protease is also an aspartic protease. Serine proteases are some of the most numerous, with some 18,000 identified across all species, and include 178 human proteases. The most important of these are some of the proteases of the clotting cascade, degradative enzymes such as elastase and dipeptidyl peptidases such as DPP-IV. The 148 human cysteine proteases also include a number of drug targets of

significance, including the caspases and most of the cathepsin. The 186 metalloproteases form another large family of proteases, which includes dipeptidases, such as angiotensin-converting enzyme (ACE), and various classes of matrix metalloproteases (MMPs), such as gelatinase, collagenase, and aggrecanase. The majority of the 28 threonine proteases are intracellular enzymes, with the largest subfamily comprising 19 proteasome subunits that are located in the cell nucleus.

The commercial potential of protease inhibitors has been most clearly demonstrated by ACE inhibitors and HIV protease inhibitors. ACE inhibitors are firmly established as the leading class of antihypertensive agent, with many of them achieving blockbuster status. HIV protease inhibitors have made a substantial contribution to the treatment of HIV infection, providing the second major class of drugs used in its treatment. Other commercially successful protease inhibitors include the matrix metalloprotease inhibitor doxycycline, the beta-lactamase inhibitor clavulanic acid, and the proteasome inhibitor bortezomib (Velcade).

2007 saw two new classes of protease inhibitors with significant commercial potential become established. Two DPP-IV inhibitors became available for the treatment of type II diabetes, and the first renin inhibitor became available for the treatment of hypertension. Merck quickly established sitagliptin (Januvia) as a significant diabetes treatment product, with sales of \$668 million in 2007, while uptake of Novartis' vildagliptin has been hampered by regulatory issues. In March 2007, Novartis launched aliskiren in the United States as Tekturna, but sales uptake has been slower.

Some 150 protease inhibitors are reported to be in clinical or preclinical development, but approximately 50% are being developed by companies other than the major pharmaceutical companies. Cardiovascular indications account for one-third of all protease inhibitors in development. Antithrombotic agents account for 38 of these, with a further 12 agents being developed for the treatment of hypertension. Diabetes, Alzheimer's disease, and hepatitis C infection are the three other major indications for which protease inhibitors are being developed.

Most of the protease inhibitors being developed for the treatment of hypertension are renin inhibitors, with Merck and Actelion's MK-8141 and Novartis' SPP-635 both in Phase II studies. The antithrombotic agents in development fall into three major groups, the direct factor Xa inhibitors, the thrombin inhibitors, and indirect factor Xa inhibitors that are more comparable to low-molecular-weight-heparins. October

2008 saw Bayer's factor Xa inhibitor rivaroxaban (Xarelto) launched in Europe, while the NDA was filed in July 2008. Bristol-Myers Squibb's apixaban commenced Phase III studies in 2008, but preliminary data from a Phase II study suggested that it had not demonstrated non-inferiority to enoxaparin, in contrast to results reported for rivaroxaban. The indirect factor Xa inhibitor fondaparinux (Arixtra) has been available since 2002, and three other such agents are in Phase III studies, with NDA submission for the most promising of these, sanofi-aventis' idrabiotaparinux, currently scheduled for 2009. Boehringer Ingelheim's thrombin inhibitor dabigatran (Pradaxa/ Rendix) was launched in August 2008 in Europe, with no other thrombin inhibitors expected to reach the market before 2013. AstraZeneca's AZD-0837 commenced Phase III studies in 2007.

Following the successful launch of the first DPP-IV inhibitors, a further 22 are now in clinical development, as well as a number of combination products. 2008 has seen NDA filings for Takeda's alogliptin, both alone (in January) and in combination with pioglitazone (in September), and for Bristol-Myers Squibb's saxagliptin in July. Both Boehringer Ingelheim's linagliptin and Phenomix' dutogliptin commenced Phase III studies in 2008.

The two proteases that regulate the production of amyloid-precursor protein, beta-secretase and gamma-secretase, account for much of the interest in Alzheimer's disease. Two gamma-secretase inhibitors are in advanced development, while development of a third, Myriad's tarenflurbil, was discontinued in 2008 after poor efficacy in Phase III studies. Eli Lilly's semagacestat commenced a four-year Phase III study in March 2008, highlighting the slow pace of development for this indication. Two other protease inhibitors are in Phase II studies for CNS disorders, Solvay's dual endopeptidase inhibitor SLV-334 for traumatic brain injury and D-Pharm's MMP inhibitor DP-b99 for stroke.

None of the protease inhibitors currently in development for the treatment of inflammatory and musculoskeletal diseases have yet progressed beyond Phase II studies. Targeted proteases include chymase, for asthma and atopic dermatitis (and also for congestive heart failure); elastase, for chronic obstructive pulmonary disease (COPD) and cystic fibrosis; cathepsin S, for rheumatoid arthritis; MMP-9, for dry eye disease; MMP-12, for COPD; MMP-13, for arthritis; and aggrecanase, for osteoarthritis. In contrast, three cathepsin K inhibitors have progressed to at least Phase II studies for osteoporosis; Medivir's MIV-701 and Ono's ONO-5334 are in Phase II while Merck's odanacatib commenced Phase III studies in 2007, with an NDA filing anticipated in 2012.

Few protease inhibitors are currently being developed for the treatment of cancer, with three metalloprotease inhibitors in clinical development as well as odanacatib for the treatment of bone cancer. Ambrilia's MMP-9 inhibitor tigapotide is being developed for prostate cancer, Incyte's ADAM10/ADAM17 inhibitor aderbasib for breast cancer, and Exelixis' ADAM10/MMP-2 inhibitor XL-784 for diabetic nephropathy.

Activity in the antiviral field has now switched from HIV to hepatitis C, although one HIV protease inhibitor, Narhex' DG-17, is in Phase II studies. Conatus' MMP inhibitor CTS-1027 is one of six agents in advanced development for hepatitis C and is currently in Phase II studies, as is LG Life Sciences/Gilead's caspase inhibitor LB-84451. Two of the four NS3a protease inhibitors are now in Phase III studies, while Merck's MK-7009 and Medivir/Tibotec's TMC-435350 are in Phase II. Schering-Plough's boceprevir commenced Phase III studies in August 2008 while Vertex' telaprevir commenced a 1,050-patient study in March 2008. Phase II data also suggest that telaprevir is the more effective of the two as an antiviral agent.

These developments indicate that more protease inhibitors will reach the market in the near future, with the most substantive development coming from the availability of new classes of oral antithrombotic agents that could supplant warfarin. The availability of better-tolerated, oral, anti-hepatitis C agents is further away, but results to date suggest that this will provide a major medical advance. A further breakthrough could come in the treatment of Alzheimer's disease, although success with the latter target could prove more problematic. Protease inhibitors should also provide a new treatment option for osteoporosis and may subsequently do so for inflammatory disorders, while it is evident that there will soon be a number of DPP-IV inhibitors available for the treatment of type II diabetes.