



CONFERENCE REPORT

Ubiquitin Drug Discovery & Diagnostics 2009 – First Annual Conference

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The Ubiquitin Drug Discovery & Diagnostics Conference, held in Philadelphia, included topics covering new therapeutic developments in the field of ubiquitin drug research. This conference report highlights selected presentations on emerging ubiquitin targets in oncology and on proteasome inhibitor therapy for the treatment of multiple myeloma. Investigational drugs discussed include MLN-4924 and MLN-9708 (both Millennium Pharmaceuticals Inc), P-005091 (Progenra Inc), CEP-18770 (Cephalon Inc) and carfilzomib (Proteolix Inc).

Introduction

The first in a new annual series of conferences, Ubiquitin Drug Discovery & Diagnostics 2009 brought together leaders from academia and industry to discuss ubiquitin and its role in drug discovery, including recent progress in emerging targets, drugs in development and diagnostic/prognostic technologies based on the ubiquitin pathway. Presentations from several key scientists who helped characterize the field of ubiquitin research were featured. This conference report highlights selected presentations on emerging ubiquitin targets in oncology and on proteasome-inhibitor therapy for the treatment of multiple myeloma.

Emerging targets in oncology

The ubiquitin-proteasome pathway (UPP) represents a promising target for disease therapeutics because of its key role in protein degradation. Many different types of cancer have been linked to the aberrant expression of enzymes in the UPP, and these associated enzymes are therefore particularly interesting as therapeutic targets. Accordingly, several presenters discussed enzymes that are potential targets for the treatment of neoplastic disease.

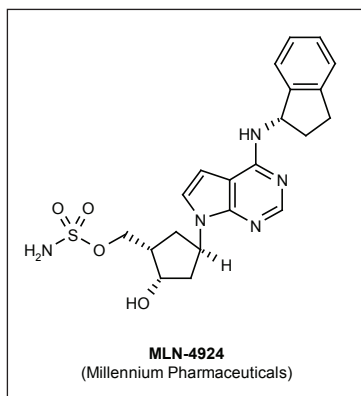
Targeting the BRCA1-associated protein 1 deubiquitylating enzyme

Keith Wilkinson (Emory University) discussed BRCA1-associated protein 1 (BAP1), a deubiquitylating enzyme (DUB) that associates with BRCA1 and has been demonstrated to be a tumor suppressor. BAP1 enhances the growth-suppressive activity of BRCA1 and localizes to the nucleus. The tumor-suppressing activity of BAP1 requires both the catalytic deubiquitylating activity of the enzyme and the C-terminus of the protein for nuclear localization. BAP1 also contributes to the control of

the G₁/S transition in the cell cycle, and inhibiting the enzyme therefore may be therapeutically beneficial. Specifically, BAP1 associates with host cell factor 1 (HCF-1), a regulator of the cell cycle checkpoint for the G₁/S transition. BAP1 was proposed to prevent the degradation of HCF-1 by deubiquitylation, thus stabilizing cellular levels of HCF-1. The half-life of HCF-1 is identical in several similar lung cancer cell lines with normal levels of BAP1 (Hop62 and Hop92) and in a cancer cell line that is null for BAP1 (NCI-H226), suggesting that BAP1 is not involved in regulating the levels of HCF-1. To identify an inhibitor of BAP1, a small-molecule library was screened using ubiquitin-AMC (a fluorogenic substrate for DUBs), which led to the identification of TG2-103-1, for which preliminary SAR data have been obtained. Dr Wilkinson concluded that DUBs were druggable, and that DUB inhibitors may be useful tools for further drug development.

MLN-4924: An E1-activating enzyme inhibitor

James Brownell from Millennium Pharmaceuticals Inc (a subsidiary of Takeda Pharmaceutical Co Ltd) introduced the company's MLN-4924 (Figure 1), a potent and selective inhibitor of the ubiquitin-like protein (UBL) NEDD8 E1-activating enzyme (NAE). UBLs are similar to ubiquitin, sharing common structural features as well as a similar multistep conjugation mechanism that links them to a target protein. These post-translational modifications can alter protein function, assembly and/or localization. Disruption of the pathway can be detrimental to the viability of a cell and, thus, proteins in this pathway can be targeted for the development of cancer therapeutics. A family of E1 enzymes activates ubiquitin and UBLs in an ATP-dependent manner that initiates the multistep conjugation mechanism. To disrupt the first step in the UBL conjugation pathway, researchers at Millennium identified a mechanism to inhibit the E1 enzymes

Figure 1. The structure of MLN-4924.

selectively. E1-activating enzymes can form UBL-inhibitor adducts when exposed to small-molecule analogs of adenosine sulfamate. MLN-4924 acts as a mechanism-based inhibitor of the NAE, creating a NEDD8-MLN-4924 adduct that is catalyzed by the NAE. The binding of the NAE to the adduct blocks the active site of E1, resulting in potent inhibition of the NEDD8 conjugation pathway. MLN-4924 is in phase I clinical trials for the treatment of cancer.

Inhibitors of USP7 deubiquitylating activity

USP7, also known as herpes-associated-ubiquitin-specific-protease (HAUSP), is a DUB that regulates the levels of the oncoprotein HDM2 and its substrate, the tumor suppressor p53. The inhibition of USP7 may lead to the downregulation of HDM2, stabilizing p53 and thereby promoting apoptosis. USP7 has additional substrates, such as the adaptor protein claspin, that provide a mechanism for the p53-independent activity of USP7 inhibitors. Benjamin Nicholson from Progenra Inc discussed the company's series of selective, potent inhibitors of USP7 deubiquitylating activity, including P-005091 (P-5091). Progenra developed an assay-screening platform based on proprietary CHOP technology, which enables quantification of the protease activity of DUBs. The platform employs a reporter enzyme fused to the C-terminus of ubiquitin. This reporter enzyme is catalytically inactive when fused to ubiquitin; following site-specific cleavage by a DUB, the enzyme is released and can cleave its substrate, generating a fluorescent signal. Using the CHOP assay platform, P-005091 was identified from the screening of a small-molecule, diversity-based library. P-005091 accelerated the degradation of HDM2, while analogs of the compound induced dose- and time-dependent increases in the protein levels of p53 and p21. The P-005091 series of compounds also induced apoptosis in numerous cancer cell lines, including both p53^{+/+} and p53-mutant cancer cell lines. P-005091 was demonstrated to downregulate claspin and phosphorylation of the DNA checkpoint kinase Chk1, and to synergize with genotoxic agents. Dr Nicholson indicated that medicinal chemistry and preclinical development were ongoing for the series, with the goal of identifying a candidate for clinical development in 2010.

Frederic Colland from Hybrigenics SA described the validation and small-molecule targeting of USP7. An RNAi-based genomic screen of human USPs identified USP7 and USP8 as DUBs involved in cancer-relevant cellular models. USP7 knockdown exhibited antiproliferative activity and induced cell cycle arrest, as well as apoptosis. The inhibition of USP7 also destabilized HDM2 and resulted in the nuclear exclusion and inactivation of the antiproliferative transcription factor FOXO4. Two selective USP7 inhibitors were identified in the high-throughput screen: HBX-19818 and HBX-28258. Treating cells with either of these inhibitors induced the destabilization of Mdm2 and G₁ cell cycle arrest, and triggered apoptosis. In addition to the discovery of selective inhibitors of USP7, several ubiquitin-specific proteases were identified as potential targets for cancer therapy. From the series, HBX-41108 (Hybrigenics) was selected for further investigation.

Modulating the Wnt pathway

The Wnt pathway has been associated with many cellular pathways, particularly embryogenesis and cancer. Given the critical role of the pathway, a complex network of proteins tightly controls and regulates Wnt signaling. Axin is a negative regulator of the Wnt pathway, and mutations in the Axin gene have been linked to colon and hepatic cancer. Stephane Angers (University of Toronto) reported that Axin may also be a positive regulator of the Wnt pathway. Axin localizes to the cytoplasm of normal cells, but can undergo nuclear-cytoplasmic shuttling; activation of the Wnt pathway correlates with Axin localization to the nucleus. To establish the proteins involved in the regulation of the pathway, a gel-free tandem-affinity purification strategy was used to identify proteins that form complexes with Axin. USP34, a DUB that regulates Axin stability as well as the nuclear localization of the protein, was demonstrated to be an activator of the pathway. Knockdown studies using USP34 siRNA demonstrated that the DUB may be an attractive drug target for treating diseases in which the Wnt pathway is aberrantly activated.

Proteasome inhibitor therapy for the treatment of multiple myeloma ***Bortezomib: The first approved reversible ubiquitin proteasome inhibitor***

Inhibiting the 26S proteasome has been clinically validated as a therapeutic strategy for the treatment of multiple myeloma (MM), with disruption of the proteasome causing cytotoxic effects in cancer cells. Kenneth Anderson (Dana-Farber Cancer Institute) presented an overview of the current status of proteasome inhibitors. Mechanisms mediating the anti-MM activity of bortezomib (Velcade), which was the first reversible ubiquitin proteasome inhibitor approved by the FDA for the treatment of MM, include the induction of endoplasmic reticulum stress and anti-angiogenic and anti-osteoclastic activities. As a result of treatment with bortezomib, the median survival rate for patients with MM has increased to 3 to 7 years.

While knocking down Hsp27 restores sensitivity in bortezomib-resistant cells, the overexpression of Hsp27 confers resistance in bortezomib-sensitive cells. Enhanced cytotoxicity has been demonstrated when bortezomib therapy was used in combination with other drugs, such as tanespimycin (KOS-953; Kosan Biosciences Inc/Institute of Cancer Research UK/National Cancer Institute), perifosine (KRX-0401; Aeterna Zentaris Inc/Keryx Biopharmaceuticals Inc), vorinostat (Zolinza), panobinostat (LBH-589; Novartis AG) and lenalidomide (Revlimid). In addition to bortezomib, Dr Anderson also highlighted several other agents for potential use in the treatment of MM, including the proteasome inhibitor NPI-0052 (Nereus Pharmaceuticals Inc), which is in phase I clinical trials, and the USP7 inhibitor P-005091, which demonstrated efficacy in a xenograft plasmacytoma model of MM.

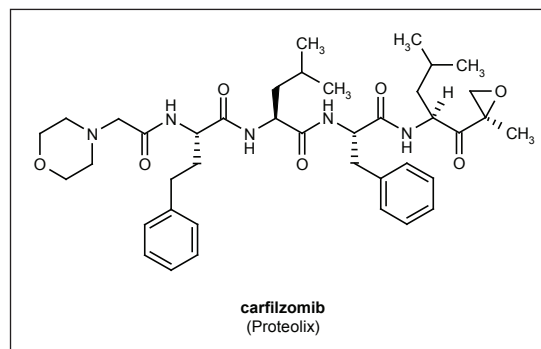
CEP-18770: A reversible boronic acid proteasome inhibitor

Bruce Ruggeri from Cephalon Inc discussed the company's orally active boronic acid proteasome inhibitor CEP-18770 (CT-18770), providing evidence for the potential use of the compound in treating MM and other malignancies that are responsive to proteasome inhibition. This reversible inhibitor has demonstrated similar antitumor activity and improved tolerability compared with bortezomib. Enzymatic and biochemical studies revealed that CEP-18770 causes inhibition of the chymotrypsin-like activity of the proteasome in the low-nanomolar range. The compound induced comparable proteasome inhibition and apoptotic profiles in xenografts of MM when administered intravenously or orally. CEP-18770 combined with melphalan or bortezomib resulted in a synergistically reduced viability of MM cells and induced apoptosis. In preclinical tumor models, CEP-18770 demonstrated superior antitumor efficacy, with substantially less cytotoxicity toward normal human epithelial cells, bone marrow progenitors and bone marrow-derived stroma cells. A reduced dose-related peripheral neuropathy was observed with CEP-18770 compared with bortezomib. Overall, the favorable characteristics of CEP-18770 compared with bortezomib support the potential use of the drug in the treatment of cancer. Dr Ruggeri indicated the compound was completing phase I clinical trials for MM, solid tumors and non-Hodgkin's lymphoma.

Carfilzomib: An irreversible ketoepoxide-based proteasome inhibitor

Mark Bennett from Proteolix Inc described research efforts to identify new proteasome inhibitors for the treatment of cancer, and discussed the company's novel irreversible ketoepoxide-based molecule carfilzomib (PR-171; Figure 2). Dr Bennett noted that the compound confers unique properties compared with other proteasome inhibitors, by binding irreversibly to the N-terminal threonine catalytic sites in the catalytic core of the proteasome; this irreversible binding of carfilzomib has been postulated to overcome resistance to bortezomib. In various

Figure 2. The structure of carfilzomib.



bortezomib-resistant tumor cell lines (eg, HT-29), as well as in CD138+ cells from bortezomib-refractory patients with MM, carfilzomib was active and able to trigger cell death. Furthermore, the irreversible binding of carfilzomib prolonged inhibition, suggesting that lower doses of the drug could be efficacious. Chronic toxicity studies in rats and monkeys demonstrated that the compound lacked neurotoxicity and did not result in neutropenia. In phase I, dose-escalation clinical trials, carfilzomib exhibited > 80% proteasome inhibition at active doses following consecutive days of dosing. In phase II trials, carfilzomib therapy was tolerated for up to 1 year in patients with relapsed and refractory MM. Dr Bennett indicated that the promising activity of carfilzomib in phase II trials provided a basis for the initiation of phase III trials, which were expected to commence in 2010.

Preclinical results for MLN-9708

Erik Kupperman (Millennium Pharmaceuticals) presented preclinical results for the company's orally active proteasome inhibitor MLN-9708. When exposed to aqueous solution, MLN-9708 is hydrolyzed to its biologically active form MLN-2238; all of the preclinical results presented were obtained using MLN-2238, which was demonstrated to exert systemic pharmacological effects. MLN-2238 binds the β_5 subunit of the 20S proteasome, causing inhibition and potent activity in cultured cancer cells. MLN-2238 demonstrated equivalent *in vitro* activity when compared with bortezomib, but with a shorter half-life for the inhibition of the proteasome. The shorter half-life was hypothesized to improve tissue distribution, because the covalent bond formed with MLN-2238 resulted in faster k_{on} and k_{off} rates compared with those of bortezomib. *In vivo*, MLN-2238 exhibited improved tumor exposure in mice, as well as improved plasma exposure in rats when compared with bortezomib. In multiple xenograft models (eg, CWR22, WSU, PHTX-22Land PHTX-22C), MLN-2238 demonstrated strong antitumor activity and improved pharmacodynamics and pharmacokinetics compared with bortezomib. MLN-9708 is in phase I clinical trials for the treatment of lymphoma and solid tumors.

Summary

The important cellular functions of ubiquitin and UBLs have resulted in considerable activity in the field of

ubiquitin research. The UPP represents a promising target for disease therapeutics because of the key role of the pathway in modulating protein functions and protein degradation. Many diseases have been linked to the aberrant activity of enzymes in this pathway, highlighting the potential importance of these proteins as therapeutic targets. However, most of these enzymes still lack validation as *bona fide* therapeutic targets. In contrast, the UPP has been validated as a target with the successful development of the proteasome inhibitor bortezomib for the treatment of MM; however, toxicities and resistance have been observed with this therapy,

suggesting the need for more selective targets. Therefore, pharmaceutical companies are developing novel inhibitors of the proteasome that may exhibit improved characteristics compared with bortezomib. Driven by the need for more selective, less toxic agents, several research groups are also investigating pathways upstream of the proteasome, with a focus on specific inhibitors such as BAP1, USP7 and the NAE. There is a general consensus that the field of ubiquitin drug research is poised for rapid expansion, and that ubiquitins could eventually rival the importance of kinases in drug discovery.