

TIDES 2011 - IBC'S OLIGONUCLEOTIDE AND PEPTIDE, RESEARCH, TECHNOLOGY AND PRODUCT DEVELOPMENT CONFERENCE (PART I)

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SUMMARY

The 2011 Annual Meeting of the American TIDES conference brought together many pharmaceutical and academic scientists from the areas of oligonucleotide and peptide research. Due to overwhelming demand, a new parallel session entitled "Peptide Discovery and Development" was launched and is the basis of this report. This session focused on cutting-edge peptide design and discovery, novel peptide selection and delivery strategies that focus on exploiting protein-protein interactions, peptides that mimic molecular scaffolds and, in particular, the development of intracellular peptides for previously "undruggable" targets. It is thought that 70-80% of human proteins fall under the category of undruggable targets, in that they cannot be reached by either of the two established drug classes: biologics and small-molecule drugs. With the overwhelming therapeutic and financial benefits that lie behind these targets, much time and money has been invested to identify novel ways to access them.

DAVUNETIDE – NEUROPROTECTION BY MICROTUBULE STABILIZATION

Bruce Morimoto (Allon Therapeutics) presented data on AL-108, an intranasal formulation of the neuroprotective peptide davunetide

(NAPVSIPQ), which reaches the brain via the systemic circulation and is currently in phase II/III clinical trials. Davunetide was developed following research on traumatic head injuries that showed that the natural response to trauma was for glial cells to produce protective neurotrophic factors. One of the neuroprotective proteins was activity-dependent neuroprotective protein (ADNP), a key protein in brain development. ADNP knockout and transgenic animals with only one ADNP knockout were generated; complete knockout resulted in death, while the transgenic animals had learning and memory deficits. Natural ADNP (which is 1,100 amino acids in length) was then used as the starting point for the development of the 8-residue davunetide. It is already well documented that for neurons to function correctly, a fully functioning microtubule network is required, not only to provide structure, but also allow the transport of cargo. Data suggest that davunetide works by restoring microtubular function via microtubular stabilization, thus restoring the cytoskeleton structure within the cells. This confirmed its ability to offer neuroprotection in vitro and led to the hypothesis that, as microtubules degenerate, there is a loss of function, such as chemical transmission, that eventually leads to cell death. Furthermore, this microtubule breakdown is involved in many neurodegenerative diseases.

A phase II proof-of-principle study was conducted in patients (N = 144) with amnesic mild cognitive impairment, a precursor to Alzheimer's disease. This randomized, placebo-controlled, double-blind trial was conducted at 17 clinical sites in the U.S. Patients were administered AL-108 (5 mg/day or 15 mg b.i.d. intranasally) for 12 weeks and then tested for learning ability and spatial working memory using a computer program. The patients were tested to see if they could recognize an object visually and then recall the same object after 12 seconds. AL-108 was shown to have an improved effect on memory after 4 weeks, lasting through 12-16 weeks. Patients with progressive supranuclear palsy (PSP), a neurodegenerative disease with a high unmet medical need, are currently being recruited to a multicenter, placebo-controlled, double-blind clinical trial. Preclinical data have shown that tau pathology is associated

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with this disease. The trial aims to investigate AL-108 in approximately 300 patients for 1 year at 47 clinical sites across the U.S., Canada, Australia, France, Germany and the U.K. It was reported that half the expected number of patients had been recruited thus far. The endpoint will use laboratory tests and patient interviews.

A NOVEL TREATMENT FOR B-CELL LYMPHOMA AND LEUKEMIAS

Results for RI-BPI, a biologically potent and specific inhibitor of the transcription factor B-cell lymphoma 6 protein (BCL-6), a member of the BTB-ZF family of proteins, were presented by Ari Melnick (Weill Cornell Medical College). BCL-6 is involved in the pathogenesis of diffuse large B-cell lymphoma (DLBCL) and is thus a key target for the treatment of lymphoma, as well as for other tumor types. In healthy individuals, the body protects itself from disease by reshuffling the antibody genes within the B-cell genome, forming new mutant antibodies to fight newly acquired infections and foreign organisms. One of the proteins involved in this function is BCL-6, which makes it possible for B cells to become activated when antigens are presented to them by the T cells. Following the shuffling in the germinal center, B cells are differentiated into antibody-producing plasma cells and memory cells. However, BCL-6 must be “shut off” for this step to occur. In DLBCL, BCL-6 remains constitutively active and this last step does not occur. The protein consists of a protein interaction BTB domain at the *N*-terminus, an RD2 domain and a zinc finger domain at the *C*-terminus. BCL-6 activity influences many signaling pathways, modulating DNA damage sensing. In order to repress further transcription, the BTB domain binds the cofactor SMRT (silencing mediator of retinoic acid and thyroid hormone receptor), to form a BTB domain–SMRT complex. The BCL-6 peptide inhibitor RI-BPI was shown to be a stable, specific and potent inhibitor. Preclinical tests have shown positive results for RI-BPI, with no toxicity in mice, suppression of BCL-6-dependent DLBCLs in vivo, killing of primary human lymphoma and leukemia cells, and eradication of B-cell lymphomas and leukemia in vivo. Data were also presented that demonstrated the ability of BCL-6 to mediate resistance to imatinib (Gleevec®), and that RI-BPI disabled this resistance and led to eradication of drug-resistant cells. In combination with imatinib, RI-BPI inhibited stem cells, which are accountable for approximately one-third of all cases of acute lymphoblastic leukemia. Currently, FDA approval is awaited prior to launching clinical trials for B-cell lymphomas and leukemias.

AN UPDATE ON CBP-501

Takumi Kawabe (CanBas) presented research on the cell-penetrating peptide CBP-501, a synthetic D-amino acid peptide currently in phase II clinical trials in combination with cisplatin/pemetrexed. This multicenter study is taking place in the U.S., Canada, Russia and South America, and is investigating CBP-501 in non-small cell lung cancer (NSCLC) and malignant pleural mesothelioma. The drug has been shown to have a dual mechanism of antitumor action: it abolishes the G₂ checkpoint during the cell cycle and selectively concentrates the chemotherapeutic drug cisplatin in the tumor cells. An overview of the cell cycle was provided. Of the two checkpoints in place to prevent further production of DNA-damaged cells (G₁ and G₂), normal cells rely on the G₁ checkpoint, while cancer cells can only rely on the G₂ checkpoint because of the loss of the G₁ check-

point. Thus, research at CanBas has focused on the G₂ checkpoint during cell proliferation, and CBP-501 was identified using the company's proprietary phenotypic screening, which selects for G₂ checkpoint abrogators. CBP-501 has been shown to inhibit multiple pathways, which results in reduced phosphorylation (on Ser216) of the M-phase inducer phosphatase 3, encoded by the gene *CDC25C* and involved in regulating cell division. The combination of CB-501 with cisplatin resulted in a marked increase in the concentration of intracellular platinum in human colon cancer cell lines (HCT 116), renal cancer cell lines (ACHN), pancreatic cancer cell lines (MIA PaCa-2) and lung cancer cell lines (NCI-H226) compared with cisplatin alone. This increase in platinum concentration was found to be linked to the inhibition of the calcium-binding protein calmodulin. CBP-501 inhibited calmodulin with approximately 80- and 4-fold greater potency, respectively, than the calmodulin inhibitors calmidazolium and melittin, thus sensitizing tumor cells to cisplatin and bleomycin. The marked increase in platinum concentration led to elevated G₂/M accumulation, thus preventing cells from entering mitosis. Phase I studies showed promising signs, with no potential cisplatin-related adverse events (only redness and itching, which are easily managed) and promising CB-501/cisplatin activity in mesothelioma, ovarian cancer and NSCLC. Phase II trials are ongoing and are expected to complete enrollment this year.

PEPTIDE DISCOVERY – CIS DISPLAY

Isogenica's alternative to the industry standard phage display, CIS display, was presented by the company's Chris Ullman. Although phage display has had great success, there are limitations; it is laborious and the transformation process is inefficient, often taking months to construct libraries of 10¹⁰ particles. CIS display is an alternative “molecular evolution” process to phage display that creates much faster hits and yields active candidates within days. It is an acellular in vitro display technology and, similarly to others in this category, it overcomes the transformation problem by completely avoiding the step. Using the transcription and translation processes of *Escherichia coli* and avoiding cloning, CIS display can rapidly generate larger libraries of > 10¹³ particles, making it an excellent discovery engine for peptides and other protein scaffolds. Using the *cis*-activity of the DNA replication initiator protein RepA, CIS display can express libraries in vitro into stable protein–DNA complexes. These complexes then undergo affinity selection before the eluted complexes are regenerated by polymerase chain reaction (PCR). After three to five successive rounds, optimal ligands are identified and these are then optimized using an in-house lysate. This discovery tool has huge potential in the construction of new peptides and other protein scaffolds, G protein-coupled receptor (GPCR) agonists and antagonists, protease and kinase inhibitors, cytokine and growth factor antagonists, and antibody epitopes.

KAI-4169 – A CASE STUDY

Derek Maclean (KAI Pharmaceuticals) gave a clinical example of a novel peptide currently in development. KAI-4169 acts as an agonist of the type C GPCR calcium-sensing receptor (CaS, CaSR) and is being investigated for the treatment of chronic kidney disease-mineral and bone disorder (CKD-MBD). Patients with this condition suffer from decreased vitamin D and calcium levels, as well as increased phosphorus levels, all of which lead to increased levels of

parathyroid hormone (PTH). CKD-MBD can result in high cardiovascular risk, vascular calcification, fractures, bone pain and, ultimately, death. There are three major clinical management goals to target the complex alteration in bone and mineral metabolism known as secondary hyperparathyroidism (SHPT); these include controlling hyperphosphatemia, maintaining serum calcium in the normal range and controlling PTH levels. As PTH may be the central mediator controlling the mineral mechanisms, KAI's strategy is to control PTH via CaS in the parathyroid gland. KAI-4169 is being developed as an intravenous bolus product to be administered three times a week, with each hemodialysis session, to early-stage renal disease patients, and also as a daily transdermal patch for predialysis patients. Both forms of administration have shown predictable, con-

sistent pharmacokinetics and sustained effects on PTH. Intravenous administration has also shown promising preclinical data indicating extra benefits, such as reduction in vascular calcification and parathyroid hyperplasia. In March 2011, early-stage renal disease patients were initiated into a double-blind, randomized, placebo-controlled phase II trial, the aim of which is to study the safety, tolerability and efficacy of multiple doses of KAI-4169 administered intravenously to patients with SHPT while they receive hemodialysis.

DISCLOSURES

The author states no conflicts of interest.

The website for this meeting can be found at: <http://www.ibclife-sciences.com/TIDES/overview.xml>.