We have developed a series of PARP inhibitors, exemplified by ABT-888, that potently inhibit PARP as chemosensitizing agents. In combination studies, ABT-888 delays DNA repair induced by DNA damaging chemotherapeutics. At the same concentrations, ABT-888 potentitates the cytotoxicity of these agents in human tumor cell lines. The potentiation was demonstrated with a wide range of agents including TMZ, Irinotecan, platinum, etc.. The delay in DNA repair and the potentiation of cytotoxicity by DNA damaging agents are modulated by genetic defects on DNA repair pathways. ABT-888 has excellent PK properties and can be administrated orally or parentally. It efficiently crosses blood brain barrier, making it an excellent candidate to combine with TMZ for treating intracranial tumors. ABT-888 is well tolerated in animal models. ABT-888 inhibited PAR formation in tumors from the treated animals, which is used as a biomarker for ABT-888. Treatment of ABT-888 enhances tumor growth inhibition by a variety of chemotherapeutic agents such as TMZ, cisplatin, carboplatin, cyclophosphomide, etc. in multiple xenograft models. ABT-888 was also shown to potentiate the antitumor efficacy of TMZ in a rat 9L glioma orthotopic model. ABT-888 is currently in clinical trials. We will disclose the structure of ABT-888 at the AACR meeting.

**#1458 MG-132 sensitizes TRAIL-resistant prostate cancer cells by activating c-Fos/c-Jun heterodimers and repressing c-FLIP(L).** Wenhua Li, Xiaoping Zhang, Aria F Olumi. *Division of Urologic Surgery, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA.*

Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is a promising anti-cancer agent since it induces apoptosis in cancer cells but not in normal cells. Unfortunately, some cancer cells develop resistance to TRAIL-induced apoptosis. Therefore, it is clinically important to determine the molecular mechanisms that differentiate between TRAIL-sensitive and -resistant tumors. Previously, we have shown that the anti-apoptotic molecule, c-FLIP(L) (cellular-FLICE-inhibitory proteins long isoform), is necessary and sufficient to maintain resistance to TRAIL-induced apoptosis. We have found that c-FLIP(L) is transcriptionally regulated by the AP-1 family member protein, c-Fos. Here we report that MG-132, a small molecule inhibitor of the proteasome, sensitizes TRAIL-resistant prostate cancer cells by inducing c-Fos and repressing c-FLIP(L). c-Fos, which is activated by MG-132, negatively regulated c-FLIP(L) by direct binding to the putative promoter region of the c-FLIP(L) gene. In addition to activating c-Fos, MG-132 activated another AP-1 family member, c-Jun. We show that c-Fos heterodimerizes with c-Jun in order to repress transcription of c-FLIP(L). Therefore, MG-132 sensitizes TRAIL-resistant prostate cancer cells by activating the AP-1 family members, c-Fos and c-Jun, which in turn repress the anti-apoptotic molecule, c-FLIP(L).

**#1459 Anticancer activities of proteasome inhibitor MG132 on human malignant pleural mesothelioma cells.** Bao-Zhu Yuan, Joshua A Chapman, David Lowry, Steven H Reynolds. *Nat'l Institute for Occupational Safety & Health, Morgantown, WV.*

Malignant pleural mesothelioma (MPM), caused mainly by occupational or environmental exposure to asbestos, is an aggressive malignancy with a very poor prognosis. The general resistance of MPM to current therapeutic modalities and an ongoing increase in the incidence of MPM demonstrate the need for new treatments for this deadly disease. Proteasome inhibitors have emerged as a category of promising reagents for treating human cancers. MG132, the most commonly used proteasome inhibitor, has demonstrated its ability to induce apoptosis in some types of human cancer. In our initial attempt to determine the therapeutic effect of the proteasome inhibitors on human MPM, we chose MG132 as a prototype of the proteasome inhibitors and characterized its anti-cancer effects on NCI-H2502 and NCI-H2452, two human thoracic MPM cell lines. It was observed that as low as 0.5$\mu$M MG132 caused significant cell death in both MPM cell lines. Cell death derived mainly from apoptosis as evidenced by the appearance of a pre-G1 peak in flow cytometry analysis and propidium iodide staining. It was also observed that MG132-induced apoptosis in both MPM cell lines was accompanied by cleavage of the PARP and caspase 3 and 7 proteins. Treatment with either casapse 3 inhibitor or Z-VAD, a broad spectrum caspase inhibitor, resulted in inhibition of MG132-induced apoptosis, suggesting that MG132-induced apoptosis operates through a caspase-dependent mechanism. Further demonstration of caspase 9 activation, which is likely the consequence of MG132-induced Bid protein activation and the subsequent release of the Smac/Diablo protein from the mitochondria, suggests that a mitochondria-caspase 3 apoptosis pathway is responsible for mediating MG132-induced apoptosis in MPM cells. Analysis of MG132's effect on invasion by MPM cells, as tested by an *in vitro* Matrigel invasion assay, demonstrated that 0.1$\mu$M MG132, which showed no effect on apoptosis induction, significantly inhibited invasion by both NCI-H2452 and NCI-H2502 cells. MG132-inhibited tumor cell invasion was found to be associated with reduced Rac1 activity. These observations demonstrate proapoptotic and anti-invasion activities human MPM cells and lead to the suggestion that MG132 may be a for clinical treatment of human MPM.

**#1460 MS-275 up-regulates FAS (CD95/APO1) expression caspase-8 -mediated apoptosis in medulloblastoma cells.** Doll Neeta S. Kang, Johannes Wolff, Vidya Gopalakrishnan. *University Anderson Cancer Center, Houston, TX.*

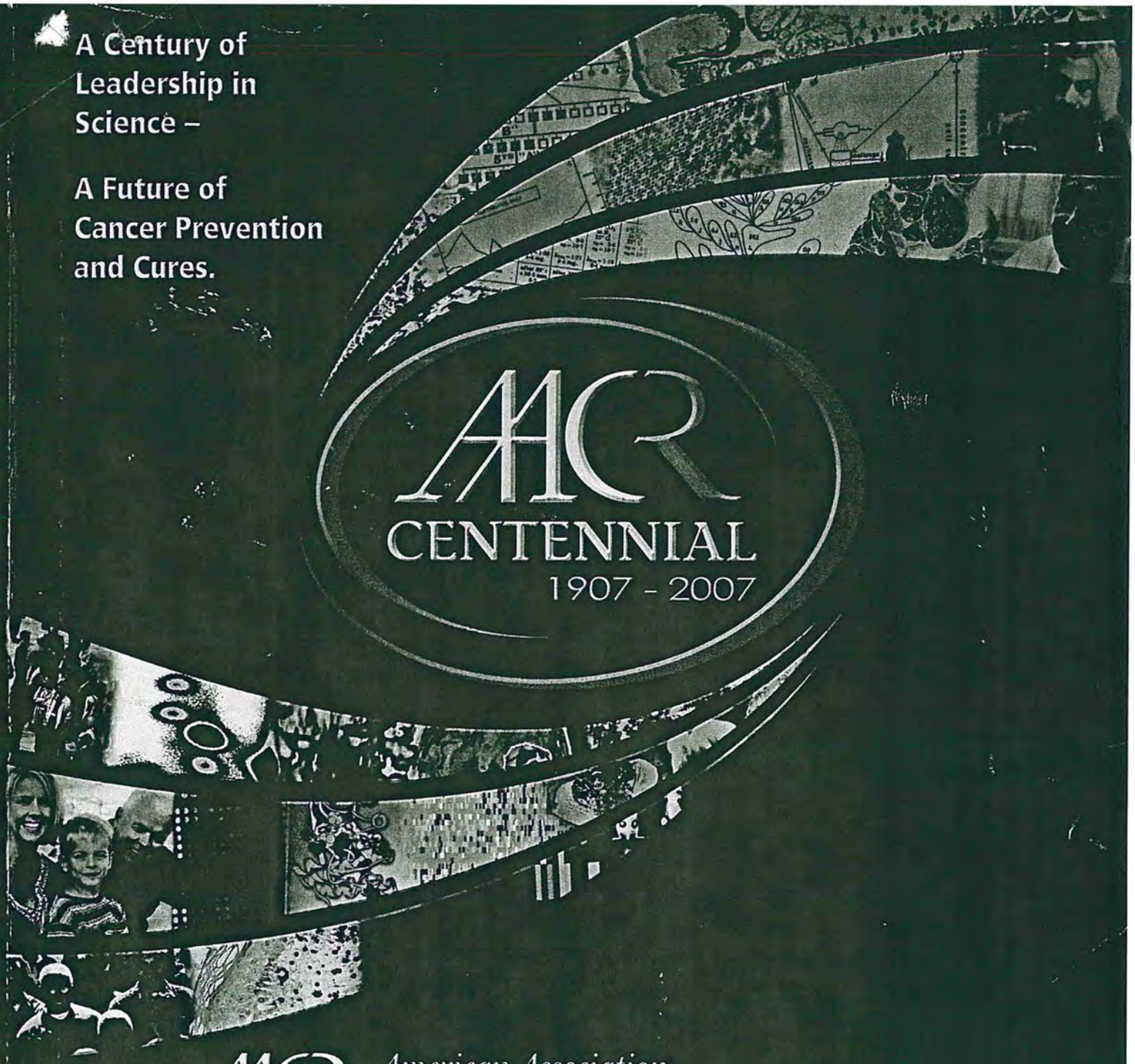
Histone deacetylases (HDACs) are key enzymes that modulate ch ture and regulate gene expression in cells by deacetylation of co residues in histones. Since aberrant expression of genes involved ir differentiation and apoptosis is an important mechanism of tumori ous cancers, we examined the effect of the histone deacetylase inhib MS-275, on medulloblastoma cell viability and gene expression *in t* onstrate that medulloblastoma cell lines, Daoy and D283, are sensit treatment and exhibit a rapid accumulation of cells with a sub-G1 This is accompanied by an increase in the expression and activity Activation of caspase-3 as well as an induction of poly ADP-ribo (PARP) cleavage was also observed as early as 24-48 hours post-t MS-275. To further confirm the involvement of the extrinsic pathwa apoptosis in response to MS-275, we measured the expression of the FAS/Apo-1 by real-time PCR, western blotting and immunofluor These experiments revealed that MS-275 not only increased the exp receptor, but also promoted a clustering of receptors as evidenced b nostaining of FAS receptor on the surface of MS-275 treated cells. FAS ligand expression remained unchanged following drug treatme appear to be necessary for MS-275 induced apoptosis, providing furt oligomerization of FAS receptors as a possible mechanism by which motes cell death. Finally, the introduction of a dominant negative FA Daoy cells interfered with the ability of MS-275 to promote apopto first demonstration that up regulation of FAS receptor and caspase-8 MS-275 plays an important role in mediating apoptosis in medullol

**#1461 OSW-1 induces apoptosis by activation of calpains and endoplasmic reticulum cell survival mechanism.** Celia García-Pi Achanta[1], Yan Zhou[1], Weiqin Lu[1], Zhou Chen[1], Yumin Hu[1], Zl Peng Huang[1]. *[1]UT M.D. Anderson Cancer Ctr., Houston, TX and Iowa, Iowa City, LA.*

Previous studies showed that OSW-1, a naturally occurring compo isolated from the bulbs of *Ornithogalum saudersiae*, exhibited pot activity and selectivity *in vitro*. This compound was shown to effectiv cancer cells including those resistant to conventional anticancer ager a unique mechanism of action of OSW-1 not shared by the conve Biochemical analysis showed that OSW-1 also caused intracellular c bances leading to massive cell death. The current study was undertal study the mechanism of action of OSW-1 and to investigate how disturbances may influence the fate of the cells. Using calcium fluo and flow cytometry, we showed that OSW-1 caused calcium elevati centration- and time-dependant manner. To evaluate whether extrace entry might play a role in OSW-1-induced calcium elevations, biocl using calcium-free medium revealed a reduction in calcium elevatio calcium increase to 50% calcium increase in calcium-free medium) treatment, suggesting that OSW-1 may cause a disturbance in the brane that allows extracellular calcium entry. Electron microscopy that OSW-1 treatment caused severe swelling of the endoplasmic re and mitochondria, which likely contributed to a further disturban homeostasis. To examine the role of calcium increase in the cytotoxici we used a calcium chelator to deprive cells of calcium and showed that manipulation significantly reduced OSW-1-induced cell death. Sinc vations were observed after OSW-1 treatment, the role of calcium-d zymes (calpains) in mediating OSW-1-induced cell death was exami the calpain inhibitor ALLN. Co-incubation of OSW-1 and ALLN sh tion in cell death compared to OSW-1 alone. Importantly, OSW-1 radation of GRP78 (an ER protein that promotes cell survival duri Chelation of calcium by BAPTA-AM or inhibition of calpains by ALI the degradation of GRP78 and suppressed OSW-1-induced cell deat together suggest that OSW-1 exerts its cytotoxity in a calcium-depen not only by activating calcium dependant-apoptosis but also by dis portant cell protective mechanism through calpain-mediated cleavage the endoplasmic reticulum.

A Century of Leadership in Science –

A Future of Cancer Prevention and Cures.



AACR CENTENNIAL 1907 - 2007

AACR American Association for Cancer Research

# Annual Meeting 2007

**April 14-18, 2007 • Los Angeles Convention Center • Los Angeles, CA**

CME jointly sponsored by the Vanderbilt University School of Medicine and the American Association for Cancer Research.

Volume 48 • April 2007



**Proceedings**