StepOnePlus Real-Time PCR machine. The extent of cytochrome c release was quantitated using immunofluorescence and apoptosis assessed using an Attune NxT flow cytometer. Gel filtration and immunoprecipitation experiments were performed according to standard protocols. Statistical analysis was performed using a two-way ANOVA with p values: * for p≤0.05, ** for p≤0.005 and *** for p≤0.001.

**Results and discussions** Employing a rapid DSF-based assay, we screened a panel of BH3 mimetics to identify that only S63845 and to a smaller extent, A-1210477, demonstrated enhanced binding to MCL-1 that correlated with a rapid, concentration-dependent apoptosis in relevant cell lines. At higher concentrations, S63845 also appeared to weakly bind BCL-2. Furthermore, S63845 synergized with other BH3 mimetics to induce apoptosis in several cancer cell lines. However, in the colorectal HCT-116 cells, BCL-XL-regulated apoptosis required all known BH3-only members, whereas apoptosis and cellular proliferation regulated by MCL-1 appeared to occur independently of all known BH3-only proteins.

**Conclusion** The anti-apoptotic and cell survival roles of BCL-XL and MCL-1 could be distinct, as antagonising BCL-XL induced BH3-dependent apoptosis, whereas MCL-1 appeared to regulate apoptosis even in the absence of all known BH3-only proteins.

**PO-062 BAX AND BAK INTERACTION WITH THE MITOCHONDRIAL PERMEABILITY TRANSITION PORE (MPTP) IS REQUIRED FOR TAXOL-MEDIATED APOPTOSIS**

O Danwesh*, R Patel. University of Leicester College of Life Sciences, Department of Molecular and Cell Biology, Leicester, UK

10.1136/esmoopen-2018-EACR25.106

**Introduction** Microtubule Interfering Agents (MIA's), such as Taxol (Paclitaxel) are used in the treatment of cancers such as breast, ovarian and non-small cell lung cancer. MIA's arrest cells in mitosis by activating the mitotic checkpoint. Prolonged mitotic arrest results in apoptotic cell death although the precise mechanism of Taxol-induced cell death is unclear. Previous studies have shown that Taxol activates the pro-apoptotic effector proteins, Bak and Bax, which accumulate at the mitochondrial outer membrane. At the mitochondrion Bak and Bax are thought to oligomerize and/or interact with the Mitochondrial Permeability Transition Pore (MPTP) to increase the permeability of the mitochondrial outer membrane which then leads to cytochrome c release and apoptosis. In this study we have examined the requirement for Bak and Bax in Taxol-induced apoptosis and their possible interaction with components of the MPTP.

**Material and methods** Human cervical carcinoma (HeLa) cells were treated with either Bax, Bak or both Bax and Bak siRNA, synchronised and treated with Taxol (60 nM) for varying times (0–24 hour). Apoptosis was assessed using either cytokeratin 18 cleavage (M30 antibody), poly (ADP-ribose) polymerase (PARP) cleavage or activation of pro-caspases (3 and 9). Bak and Bax were immunoprecipitated using anti-active Bak (N-20) and anti-active Bax (6A7) antibodies in CHAPS lysate buffer. To identify interacting proteins, Bak and Bax IPs were subjected to peptide mass fingerprint analysis by mass spectrometry. Confocal microscopy was used to examine the intracellular localization of Bax and Bak following Taxol treatment.

**Results and discussions** The results of our siRNA studies indicate that although Bax and Bak can form homo-oligomers in the absence of each other, both proteins are required for Taxol-induced apoptosis. Our immunofluorescence study shows that Bak and Bax co-localise at mitochondria in the Taxol-arrested cells. Our proteomic and co-IP analyses indicate that Bak and Bax form a complex specifically in the Taxol-arrested cells that also includes components of the MPTP such as the voltage dependent anion channel (VDAC), the adenine nucleotide translocator (ANT2) and Bcl-2.

**Conclusion** We conclude that the oligomerisation of Bax and Bak is insufficient for Taxol-mediated apoptosis. However, the interaction of activated Bax and Bak with the MPTP appears to be necessary for Taxol-induced apoptosis.

**PO-063 TRITERPENOIDS ISOLATED FROM NATURAL PRODUCT REGULATES TNF(TUMOUR NECROSIS FACTOR)-MEDIATED RIP(RECEPTOR-INTERACTING SERINE/THREONINE-PROTEIN KINASE)1-DEPENDENT APOPTOSIS**

1K Kang*, 1SR Lee, 2X Piao, HS Byun*1SR Lee, 3M Won, 3KA Park, 3GM Hur. 1College of Medicine- Chungnam National University, Department of Pharmacology and Medical Science, Daejeon, South Korea; 2College of Medicine- Chungnam National University, Department of Pharmacology and medical science, Daejeon, China; 3College of Medicine-Chungnam National University, Department of Pharmacology, Daejeon, South Korea

10.1136/esmoopen-2018-EACR25.107

**Introduction** TNF(Tumour necrosis factor) have important role in NF-kB(Nuclear factor kappa-light-chain-enhancer of activated B cells)-mediated inflammatory response and is a pharmacological target of cancer. Activation of TNF signalling can result in cell survival and also death pathway. However, in most cell types including cancer cells, TNF stimulation does not induces cell death. This fact reflects that TNF-mediated cell death pathway is disrupted in cancer cells, and provides TNF is unsuccessful in anti-tumour therapy as a single agent. Nevertheless, recent studies demonstrated that TNF-mediated cell death sensitises RIP1-dependent apoptosis or necroptosis in a certain conditions, thereby suggesting that regulating TNF-mediated cell death can be new therapeutic target for cancer.

**Material and methods** 1 cell culture MEF(Mouse embryonic fibroblast) cells were cultured in DMEM(Dulbecco’s modified eagle’s medium) supplemented with 10% heat-inactivated FBS(Fetal bovine serum), 2 mM glutamine, antibiotics (100 U/mL penicillinG and 100 µg/mL streptomycin), and were maintained at 37°C in a humidified incubator containing 5% CO2.

2. Immunoblot analysis Cells were collected and lysed in M2 buffer and 20 μg of the cell lysates were fractionated by 10% SDS polyacrylamide-gel and blotted onto PVDF membrane. After blocking with 5% skim milk in PBS/T, the membrane was proved with the relevant antibody and visualised by ECL(Enhanced chemiluminescence).

**Results and discussions** To identify chemical compound inducing TNF-mediated cell death, we screened triterpenoids isolated from natural product, it is already known that have anticancer effect.

Here, we propose that triterpenoids isolated from natural product induces TNF-mediated RIP1-dependent apoptosis or
necroptosis not a RIP1-independent apoptosis. Interestingly, triterpenoids did not affect the TNF-induced RIP1 ubiquitination and induces TNF-mediated cell death independently of NF-xB. These observations suggesting that triterpenoids have specific target and effect on regulating TNF-mediated cell death. Thus, triterpenoids represents a novel compound of therapeutic candidate for the treatment of cancer.

Conclusion In this study, we demonstrate that the Triterpenoids isolated from natural product induces the TNF-mediated RIP1-dependent apoptosis, and effect of Triterpenoids-induced apoptosis is regulated independent of NF-xB. Findings from this study, we suggest that a novel function of Triterpenoids as a useful anti-cancer agent.

PO-064 TUBULIN-BINDING ANTI-CANCER POLYSULFIDES INDUCE CELL DEATH VIA MITOTIC ARREST AND AUTOPHAGIC INTERFERENCE IN COLON CANCER

1E Yagdi*, 2A Mazumder, 3JY Lee, 1A Gaigneur, 2F Radogna, 1M Dicato, 2P Chaimbault, 4C Jacob, 4C Corella, 4M Diederen, 1Hôpital Kirchberg, Laboratoire de Biologie Moléculaire et Cellulaire du Cancer, Luxembourg, Luxembourg; 2Seoul National University, College of Pharmacy, Seoul, South Korea; 3Université de Lorraine, Laboratoire Structure et Ractivité des Systèmes Moléculaires Complexes, Metz, France; 4Saarland University, School of Pharmacy, Saarbrücken, Germany

Introduction Colon cancer is a major cause of morbidity and mortality worldwide. Epidemiological studies revealed an inverse correlation between colon cancer risk and a garlic-rich diet. Natural organosulfur compounds confer protective effects against a wide range of cancer, including colon. Here, we studied the anti-colon cancer activity of garlic-derived diallyl/dibenzytetrassulfur (DATTS/DBTTS) derivatives.

Material and methods We validated the ability of DATTS/DBTTS to bind tubulin by MALDI-TOF mass spectrometry and its disruptive effect on the microtubule network by immunofluorescence. We selected cell lines with a defined genetic background including HT-29 (BRAF-mutation), SW480 and metastatic SW620 (both KRAS-mutation). Cell cycle analysis, Hoechst staining and western blots allowed to assess cell death. To further validate the anti-cancer activity of DBTTS, colony and spheroid formation assays as well as zebrafish xenografts were realised. To monitor autophagy, western blots, GFP-LC3 plasmid transfection and transmission electron microscopy (TEM) were conducted.

Results and discussions All selected cell models were more sensitive to DBTTS than to DATTS. SW480 and SW620 were more susceptible to DBTTS than HT-29 cells. DBTTS induced mitotic arrest followed by cell death. Its anti-cancer activity was validated in 3D cell culture systems and in vivo. DATTS/DBTTS acted as a direct and reversible tubulin binder inducing microtubule disarrangements in cellulo. As tubulin alterations may affect autophagy progression, we evaluated the effect of DBTTS on autophagy. DBTTS induced LC3-II and p62 protein accumulation concomitantly with mitotic arrest in HT-29 but not in SW480 and SW620 cells. TEM analysis showed accumulation of pre-fusion complexes (phagophores and autophagosomes) indicating inhibition of the autophagic flux. Autophagy inhibitor bafilomycin A1 confirmed the impairment of the autophagy flux by DBTTS in HT-29 cells. Immunofluorescence revealed p62 protein accumulation showing a dotted pattern, similar to the LC3-II puncta formation in HT-29 cells. Furthermore, silencing of p62 protein exaggerated cell death indicating a pro-survival role of p62 overexpression in DBTTS-treated HT-29 cells.

Conclusion Altogether, we showed here that DBTTS acts as an anti-cancer agent by targeting tubulin. These results suggest that DBTTS targets colon cancer survival/death through autophagy interference depending on cell types with differential autophagy capacities and genetic signatures.

PO-065 ARGinine DEPRIVATION THROUGH HUMAN RECOMBINANT ARGINASE I (CO)-PEG5000 [HUArgI (CO)-PEG5000] ACTIVATES AUTOPHAGY AND LEADS TO AUTOPHAGY-MEDIATED CELL DEATH IN GLIOBLASTOMA CELLS

R Abi-Habib*, Y El-Jawhari, E Jbeily, M El-Sibai. Lebanese American University, Department of Natural Sciences, Beirut, Lebanon

Introduction Arginine auxotrophy, secondary to a lack of argininosuccinate synthetase-1 (ASS-1) expression, is a hallmark of several tumour types allowing for their selective targeting using arginine deprivation. HuArgI (Co)-PEG5000, a recombinant PEGylated human arginase I in which the two active site Mn** ions have been replaced by Co** ions, degrades extracellular arginine leading to arginine deprivation and selective cytotoxicity to GBM cells. In this study, we investigate the mechanisms of arginine deprivation-induced cell death, particularly the contribution of autophagy, in glioblastoma (GBM) cells.

Material and methods Four GBM cell lines were used to determine extent and dynamics of autophagy activation using Cyto-ID staining on fluorescent microscopy and LC3-I to II conversion using western blot. The contribution of autophagy to arginine deprivation-induced cell death in GBM cells was investigated through inhibition of autophagy, at different time points, using Chloroquine (CQ).

Results and discussions [HuArgI (Co)-PEG5000]-induced arginine deprivation led to a clear and time-dependent increase in the autophagic flux, as indicated by the increase in the number of autophagosomes on Cyto-ID and in the processing of LC3-I to LC3-II, in all cell lines, starting at 12 hours and increasing with time to peak at 96 and 120 hours post treatment. Blocking the autophagic flux using CQ, a downstream inhibitor of autophagy, led to a further accumulation of autophagosomes at all time points. Inhibiting autophagy, using CQ, had a binary, time-dependent effect on the cytotoxicity of [HuArgI (Co)-PEG5000]-induced arginine deprivation in GBM cells. At early time points (12, 24 and 48 hour), inhibition of autophagy increased cell sensitivity to arginine deprivation underlying the protective role of autophagy while at late time points (72, 96 and 120 hour), inhibition of autophagy significantly decreased or completely prevented cell death indicating that autophagy plays a deleterious role in GBM cells exposed to arginine deprivation for a prolonged period of time.

Conclusion This study demonstrates that arginine deprivation leads to significant and sustained activation of the autophagic flux in GBM cells. Our data also indicates that the observed over-activation of autophagy is protective to GBM cells at early time points but becomes deleterious at later time points leading to autophagy-induced cell death (death by autophagy), following prolonged arginine deprivation.