evidence has demonstrated statins to possess anti-cancer properties against a wide range of tumours without being toxic to normal cells. Multiple myeloma (MM) is largely incurable and in acute myelogenous leukemia (AML), less than 50% of patients with poor cytogenetic disease have a chance of long-term survival. Therefore, novel therapeutic strategies are urgently needed to treat these haematological malignancies, subsets of which are sensitive to statin-induced apoptosis. Through the use of a chemical library screen, we hypothesize that the identification of compounds which potentiate the anti-cancer effects of statins will uncover novel molecular pathways and/or targets that can be exploited in combination with the MVA pathway to maximize tumour cell death in MM and AML.
A pilot 100-compound library, composed of off-patent pharmacologically active drugs clinically used for a wide spectrum of diseases was screened in the MM KMS11 cell line. Dipyridamole (DP), a commonly prescribed anti-platelet agent potentiated the anti-cancer effects of atorvastatin. The DP-statin combination is synergistic and capable of inducing apoptosis in a variety of AML and MM cell lines as well as primary AML patient samples. DP is a wide-acting agent and known to elicit numerous effects at the molecular and global physiological level. Further investigations at the level of mechanism and evaluation of *in vivo* efficacy are currently underway. As both statins and DP are pre-approved for use in humans, off-patent, and readily available, they have the potential to directly impact patient care.

85 POSTER

Mechanisms underlying the therapeutic benefit of necitumumab (IMC-11F8) in combination with cisplatin/gemcitabine in NSCLC xenograft models

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Our previous study revealed the anti-tumor benefits of adding Necitumumab, an investigational recombinant human-EGFR antibody to Cisplatin+Gemcitabine (C+G) in A549 and NCI-H1650 subcutaneous xenograft models established in nu/nu mice. The present study utilized qPCR Arrays to evaluate 340 pathway specific genes to explore the mechanisms underlying the combination benefits. Significantly affected human tumor mRNAs, either up or down regulated by 2 fold with $p < 0.05$ versus control (t-test, n = 3), were identified and processed utilizing Ingenuity Pathway Analysis to explore the cell functions associated with the mRNA effects of treatment. Histological analysis of tumor sections was performed to support this analysis.
Combination therapy again significantly inhibited the growth of xenograft tumors compared to either necitumumab alone or chemotherapy alone in both models. In A549, necitumumab+C+G increased BCL2L11 (69.2 fold, $p = 0.0019$), PYCARD (20.9 fold, $p = 0.02$), CARD8 (36.8 fold, $p = 0.02$), CARD6 (12.7 fold, $p = 0.00036$), CDKN2A (66.7 fold, 0.004), APC (17.3 fold, $p = 0.005$), RARB (9.0 fold, $p = 0.007$), RXRA (4.3 fold, $p = 0.03$), BFAR (4.1 fold, $p = 0.04$), CD40 (5.3 fold, $p = 0.03$), CD40LG (36.79 fold, $p = 0.02$) and CASP9 (164.4 fold, $p = 0.008$) mRNA compared to saline, necitumumab and C+G alone. The finding that these genes are associated with apoptosis signaling is in agreement with a 5 fold increase in tumor cell apoptosis detected by IHC. On the other hand, combination treatment in the NCI-H1650 model increased GADD45A (3.25 fold, $p = 0.0061$), CDKN1A (6.1 fold, $p = 0.031$), BRCA2 (3.54 fold, $p = 0.096$), BID (2.4 fold, $p = 0.05$), BNIP3 (−2.2 fold, p-0.02), HSF1 (23.6 fold, $p = 0.016$), IGFBP3 (26.6 fold, $p = 0.03$) expression, and down-regulated BCL2 (-67.5 fold, $p = 0.0013$), CCND1 (-14.4, $p = 0.0007$), FOXA2 (−7.4 fold, $p = 0.0085$), IL2 (−7.4 fold, $p = 0.008$), LEF1 (−8.01 fold, $p = 0.0031$), PECAM1 (−7.33 fold, $p = 0.0022$), WISP1 (−2.1 fold, $p = 0.022$), p53 (−167.2 fold, $p = 0.0003$) and CDK2 (−3.04 fold, $p = 0.024$). These genetic signature changes suggest arrest of cell growth which was again supported by histological analysis demonstrating that tumor cell expression of the proliferation marker Ki67 was decreased.
In conclusion, the mechanisms underlying the consistent antitumor benefits of necitumumab in combination with G+C in NSCLC xenograft models are variable, in that treatment differentially affected mRNA expression, proliferation and apoptosis in two different models.

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Potent anti-tumor activity of T-DM1 antibody–drug conjugate in combination with chemotherapeutic agents in breast tumor cells

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Patients with HER2-positive breast cancer who progress during trastuzumab treatment or who do not initially respond to treatment have a critical need for alternative therapy. T-DM1 is an antibody-drug conjugate (ADC) consisting of the anti-mitotic agent DM1 (a maytansine derivative) covalently linked to trastuzumab through a stable MCC linker. T-DM1 has potent anti-tumor activity in HER2-overexpressing trastuzumab-sensitive and -resistant tumor cell lines and xenograft models of human cancer. T-DM1 is currently undergoing clinical evaluation in metastatic HER2-positive breast cancer patients whose disease is refractory to HER2-directed therapies. Since breast cancer patients are treated with many chemotherapeutic agents, we investigated combinations of T-DM1 with two of these agents by measuring both the *in vitro* anti-proliferative activity in HER2-overexpressing breast tumor cells and the *in vivo* anti-tumor efficacy in breast cancer xenograft models.
Combinations of T-DM1 with either gemcitabine (Gemzar®) or docetaxel (Taxotere®) were evaluated in parallel using cellular proliferation assays and xenograft tumor models. The *in vitro* data were analyzed using the combination index method developed by Chou & Talalay while the *in vivo* data were analyzed using a method recently developed at Genentech. Using these methods, all combination effects were classified as synergistic, additive, or antagonistic. An additive effect was observed when T-DM1 was combined with docetaxel both *in vitro* and *in vivo*. An antagonistic effect was observed when T-DM1 was combined with gemcitabine *in vitro* while an additive effect was observed *in vivo*. Further analysis of this combination *in vivo* revealed that gemcitabine has a diminishing contribution to the overall effect as the concentration of T-DM1 is increased.
In summary, these studies demonstrate the potent anti-tumor activity of T-DM1 compared to and in combination with conventional chemotherapeutic agents. Furthermore, these studies provide a framework for future *in vivo* combination analysis using a multi-dose platform model.

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BEZ235, a dual PI3K/mTORC inhibitor, targets the DNA damage response leading to radiosensitization and senescence

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Background: Activation of the phosphatidylinositol 3-kinase (PI3K) signalling pathway is associated with resistance to ionizing radiation (IR) pre-clinically and in clinical studies. We investigated the effect of BEZ235, a novel dual PI3K/mammalian target of rapamycin complex (mTORC) inhibitor currently in clinical development, on the response to IR.
Materials & Methods: The effect of BEZ235 and IR was assessed *in vitro* using clonogenic survival assays and *in vivo* using subcutaneous xenografts in athymic nude mice. Effects on cell survival, DNA damage and senescence were also evaluated.
Results: Treatment with BEZ235 increased cellular radiosensitivity as evidenced by reduced clonogenic survival after IR. The D37 (radiation dose resulting in 37% surviving fraction) was reduced from 4 Gy to 1 Gy in H460 cells; from 5.2 Gy to 0.9 Gy in A549 cells; and from 3.3 Gy to 0.8 Gy in A431 cells after BEZ235 treatment. Using a clinically relevant fractionated radiotherapy protocol, we also found that tumor growth was significantly lower following treatment with BEZ235 and IR compared to IR alone in both H460 and A431 xenografts ($P < 0.05$). In addition to its effects on PI3K/mTOR signalling, BEZ235 also inhibited IR-induced activation of DNA-dependent protein kinase catalytic subunit (DNA-PKcs; *PRKDC*). This resulted in accumulation of DNA double-stranded breaks (DSBs) as evidenced by the alkaline COMET assay and persisting phospho-γH2AX foci. In turn, persistent DNA damage was associated with the induction of senescence in some models. Interestingly, a selective DNA-PKcs inhibitor (KU57788) had comparable effects to BEZ235 on the DNA damage response (DDR) and induction of senescence after IR, while a selective PI3K inhibitor (BKM120) and a selective mTORC1 inhibitor (RAD001) alone or in combination did not, indicating that DNA-PKcs inhibition is an important factor in radiosensitization by BEZ235.
Conclusion: BEZ235 enhances the *in vitro* and *in vivo* efficacy of IR. The mechanism of cellular radiosensitization due to BEZ235 involves modulation of the DDR with inhibition of DNA-PKcs leading to accumulation of IR-induced DNA DSBs. In specific cellular contexts the persisting DDR is associated with cellular senescence. These data offer a mechanistic explanation for radiosensitization by BEZ235 and provide a rationale for clinical studies of this combination.