which greatly increases drug selectivity toward cancer cells and also might allow by-passing drug cell resistance, when this is generated by mechanism of drug internalization or drug export. Moreover, modularity of drug-armed NT4 allows tailoring of drug-armed peptides on the basis of sensitivity of cancer cells to different drugs.
NT4 armed with 5-fluoro-deoxyuridine was used for *in vivo* experiments in HT-29-xenografted mice and produced a 50% reduction of tumor growth with respect to animals treated with equal amount of the un-conjugated drug.
In vitro and *in vivo* results indicated that branched peptides are valuable tools for tumor selective targeting.

81 POSTER

Potential clinical application of a novel Heat Shock Protein 90 inhibitor CH5164840: preclinical efficacy in mono therapy and combination therapy

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Background: HSP90 is a molecular chaperone and plays an important role in protein folding and stability. In tumor cells, HSP90 is activated by forming super chaperone complexes with co-chaperones. Inhibition of HSP90 function leads to degradation of multiple oncogenic client proteins, resulting in loss of signal transduction, growth inhibition, cell death, and anti-angiogenesis. This unique feature is expected to overcome the problem of resistance to TKIs. Thus, targeting HSP90 is considered to be an attractive strategy for anticancer therapy.
Results: We have identified CH5164840 as an HSP90 inhibitor with a novel chemical structure through virtual screening based on 3D-structure. CH5164840 binds to an ATP-binding pocket of HSP90 comparable to that of ansamycins, 17-AAG and 17-DMAG. Treatment with CH5164840 showed marked degradation of multiple clients in a dose- and time-dependent manner. Consistent with its selective binding to HSP90 in the super chaperone complex, longer pharmacodynamic duration and tumor retention profiles, CH5164840 shows tumor-selective degradation in vivo and therefore exhibits potent antitumor efficacy with a wider therapeutic range in NCI-N87, a Her2 positive gastric cancer model. Further extended efficacy studies with oral daily administration of CH5164840 in many xenograft models revealed that CH5164840 is sensitive to RTK-addicted tumors that occur when EGFR and HER2 are mutated or dysregulated. Moreover in combination therapy, CH5164840 enhances the anti-tumor efficacy with current standard RTK inhibitors.
Conclusion: CH5164840 is a novel, orally available, synthetic HSP90 inhibitor and shows highly potent antitumor efficacy in mono- and combination-therapies with standard-of care-agents. These profiles support the clinical development of CH5164840 for the treatment of RTK-addicted tumors, including tumors with overexpression and mutation of RTKs whose growth and survival depend on HSP90.

82 POSTER

Pharmacokinetic–pharmacodynamic modeling of the effect of GDC-0152, a selective antagonist of the inhibitor of apoptosis (IAP) proteins, on monocyte chemotactic protein-1 (MCP-1) indicates species differences in MCP-1 response

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Inhibitor of apoptosis (IAP) proteins are believed to suppress apoptosis and are overexpressed in a variety of cancers. GDC-0152 is a potent and selective antagonist of the IAP proteins that was developed as a potential treatment of tumors that are resistant to chemotherapies or radiotherapy. Monocyte chemotactic protein-1 (MCP-1) is a chemokine that is expressed during an inflammatory response. Based upon preclinical studies, antagonism of IAP proteins has been shown to induce MCP-1 expression via cIAP degradation and activation of NF-kB signaling. The objective of this study was to investigate species differences in MCP-1 response to GDC-0152 in rats, dog, and humans using pharmacokinetic/pharmacodynamic (PKPD) modeling. Briefly, dogs (n = 40) and rats (n = 20) were given intravenous (IV) doses of GDC-0152 ranging from 0.3 to 15 mg/kg and 20 to 120 mg/kg, respectively. A two compartment model was used to characterize the pharmacokinetics of GDC-0152 in both dogs and rats. A semi-mechanistic population PKPD model incorporating transit compartments was used to characterize the MCP-1 response to GDC-0152. Estimated parameters from the described model indicate that lower concentrations of GDC-0152 are required to trigger an increase in MCP-1 plasma levels in dogs when compared to rats. Simulations were performed with pharmacodynamic (PD) parameters estimated from rat and dog using human pharmacokinetic parameters and select doses. In simulations performed using dog PD parameters, an approximately 4-fold increase in MCP-1 plasma concentration was estimated at a dose of 0.76 mg/kg. In contrast, similar simulations using rat PD parameters suggest little or no change in MCP-1. Humans given intravenous doses $\geqslant$0.76 mg/kg showed no evidence of MCP-1 increase. Thus, the dog appears to be more sensitive to GDC-0152 (in terms of MCP-1 increase) when compared to rats and humans.

83 POSTER

Effect of TG02, a kinase inhibitor targeting Erk5, on triple negative breast cancer cells

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Background: Breast cancer is the most common neoplasia in women. Mitogen-activated protein kinases (MAPK) play important roles in tumorigenesis. Formerly, we reported that one of them, Erk5, is linked to the proliferation of breast cancer cells in vitro, is commonly overexpressed in primary breast tumors, and that its overexpression is an independent negative prognostic marker for disease-free survival. In addition, inhibition of Erk5 sensitized cells to treatments commonly used in the breast cancer clinic. Therefore, Erk5 may represent a novel therapeutic target in breast cancer.
Here we studied the effect of TG02 (a multikinase inhibitor that targets Erk5) on a panel of cell lines representing the basal-like or triple negative subtype of human breast cancer (TNBC).
Materials and Methods: The expression of Erk5 in a panel of TNBC cell lines and the mechanism of action of TG02 on Erk5 were analyzed by immunoprecipitation and Western blotting, using antibodies directed against Erk5. Effects on cell proliferation were determined by MTT assay and cell cycle and apoptosis analyses were performed by propidium iodide DNA staining and FACS analysis. In vitro drug synergies were explored using a caspase 3/7 ELISA and the in vivo activity of TG02 was tested in nude mice bearing established MDA-MB-231 xenografts.
Results: The TNBC cell lines analyzed showed high levels of Erk5 expression, and Erk5 was active under resting conditions in some cases. Cell proliferation studies indicated that the TNBC cells were very sensitive to the action of TG02 at low concentrations (IC_{50} $\leqslant$100 nM) and short exposure times (24−48 hrs). TG02 also induced cell cycle arrest at the G2/M transition leading to apoptotic cell death.
As Erk5 is a target of TG02, we explored whether Erk5 activity was affected by drug treatment. The kinase activity of Erk5 was compromised even though TG02 did not affect the Erk5 upstream activating kinase Mek5, or other upstream activating kinases. In vivo studies indicated that TG02 exerted strong antitumor activity in mice bearing MDA-MB-231 xenografts as a single agent and synergized with the standard of care drug doxorubicin.
Conclusions: TNBC cells are very sensitive to TG02, both in vitro and in vivo. TG02 induced cell cycle arrest at the G2/M transition, causing cell death alone and in synergy with doxorubicin, perhaps via inhibition of Erk5. These preclinical studies establish the bases for the clinical development of this compound for the treatment of TNBC.

84 POSTER

Identifying statins and dipyridamole as a novel drug combination showing efficacy in multiple myeloma and acute myelogenous leukemia

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Statins are drugs that have been utilized for years to treat hyperlipidemia via inhibition of the rate-limiting enzyme of the mevalonate (MVA) pathway, 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR). Preclinical

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.

86 POSTER

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.

87 POSTER

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.