Conclusions: Triple knock down of cIAP-1, cIAP-2 and XIAP not only sensitised the androgen independent prostate cancer cells line, PC-3, to TRAIL induced apoptosis but also altered their proliferation rates mediated via a decrease in ID-1 expression. The inability of IAP knock down to alter type 2 triggers of apoptosis demonstrates a maintained mitochondrial staibility. IAP knock down may facilitate the bodies natural immune-surveillance mechanisms to counter cancer progression by receptor mediated apoptosis but also therapeutic approaches with TRAIL. Overcoming cancer cell resistance to therapeutic approaches represents an important combined treatment strategy.

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Synergistic anti-tumour activity of oncolytic Reovirus and cisplatin in a B16.F10 mouse melanoma model

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Background: Reovirus type 3 Dearing (RV) has demonstrated oncolytic activity in numerous in vitro systems, in vivo murine models and early clinical trials, In this study, we examine the in vitro and in vivo oncolytic activity of RV against the mouse melanoma cell line B16.F10 in combination with cisplatin (CP), a pseudoalkylating chemotherapeutic which causes DNA cross-linking and is active in a wide range of cancers.
Material and Methods: The effect of RV and CP was assessed in vitro for synergistic tumour kill and mechanism of tumour death. For in vivo evaluation, subcutaneous B16.F10 tumours in C57Bl/6 mice were treated with intratumoural RV and intraperitoneal CP either alone or in combination. Tumour volume was estimated thrice weekly. Tumours and organs were harvested post-treatment for viral retrieval and histology; serum samples were tested for induction of neutralising anti-reovirus antibody (NARA).
Results: A synergistic interaction (combination index value (CIV) of less than one) was observed between RV and CP (CIV: ED50 0.42±0.03; ED75 0.30±0.02; ED90 0.24±0.01). Flow cytometric analysis showed a marked increase in apoptotic cells following combined exposure, compared to single agent exposure. Reduced tumour growth and extended median survival time was observed in mice treated with RV/CP combination therapy compare to single agent treatments. Mean relative tumour volumes ±SD day 12 – Control all reached endpoint, RV alone 8.92±6.94, CP alone 9.87±2.80, RV plus CP 3.86±2.24. Median survival (days) – Control 6, RV 12, CP 8, Combination 17.
Live virus was recovered from the tumours of all RV only treated animals and from the liver and heart of 1/6. In contrast live virus was detected in only 50% of tumours from combination treated mice but in the liver of 4/6 mice. CP did not affect the NARA response to RV.
Conclusions: Taken together, these results indicate that the addition of chemotherapeutic agents can significantly enhance the anti-tumour efficacy of RV therapy and justify formal clinical evaluation.

Her

309 POSTER

Fully human anti-HER3 mAb U3-1287 (AMG 888) demonstrates unique in vitro and in vivo activities versus other HER family inhibitors in NSCLC models

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Background: HER3 is a member of the Human Epidermal Growth Factor Receptor (HER) family and is an important component of HER family driven tumorigenesis. Though HER3 lacks intrinsic kinase activity, it is a scaffold for PI3K/AKT signaling for the HER family via heterodimeric interactions. HER3 signaling may be a resistance mechanism for EGFR and HER2 inhibitors. We report unique in vitro and in vivo activities of U3-1287 (AMG 888), the first fully human Anti-HER3 mAb vs. current HER family inhibitors in NSCLC models. Combinations with EGFR inhibitors are also explored.
Methods: To determine the inhibition of HER3 oncogenic signaling, A549, Calu-3 and H1975 NSCLC cells were treated with up to 10 μg/ml of U3-1287 (AMG 888), C225 (Anti-EGFR), c2C4 (Anti-HER2) or control mAbs 1 hour prior to heregulin-beta (HRG) or vehicle stimulation. Since HER3 is a heterodimerization partner for HER family members, U3-1287 (AMG 888) was combined with EGFR inhibitors in vitro. To determine in vivo efficacy, mice bearing ~200 mm^3 A549 NSCLC xenografts were treated 2×/week with anti-HER or control Abs. A549 xenograft tumors were analyzed for the inhibition of pHER3 by Western blotting. The anti-tumor effects of U3-1287 (AMG 888) with an EGFR inhibitor was tested in the Calu-3 and H1975 NSCLC xenograft models.
Results: Treatment with U3-1287 (AMG 888) resulted in an inhibition of ligand-induced pHER3, basal pHER3 and pAkt in A549, Calu-3 and H1975 NSCLC cell lines, respectively. In NCI-H1975 cells, combining U3-1287 (AMG 888) with the anti-EGFR mAb C225 resulted in greater pHER3 and pAkt inhibition in vitro than with either single agent alone. Administration of U3-1287 (AMG 888) resulted in tumor stasis in the (EGFR TKI resistant) A549 NSCLC xenograft model vs control and other HER mAbs and tumor inhibition in Calu-3 and NCI-H1975 xenografts compared to IgG treated mice ($p < 0.05$). Combinations with the anti-EGFR mAb C225 resulted in tumor growth inhibition that was greater than either single agent alone in CaLu-3 ($p < 0.001$) and NCI-H1975 ($p < 0.001$) xenografts.
Conclusions: U3-1287 (AMG 888) inhibits basal and ligand-induced HER3 oncogenic signaling in NSCLC cell lines in vitro and basal pHER3 in vivo. NSCLC xenografts are sensitive to U3-1287 (AMG 888) treatment as single agent or in combination with an anti-EGFR mAb, including an EGFR TKI resistant model. These data provide preclinical evidence for the potential clinical application of U3-1287 (AMG 888) in NSCLC.

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ERBB2/HER2 proteasome–lysosome trafficking and degradation directed by polyubiquitination topology

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Activity of the overexpressed ERBB2/HER2 receptor tyrosine kinase is related to its cell surface recycling and ability to evade intracellular degradation. Attenuated signaling and receptor degradation may be induced by trafficking that directs internalized receptor to either the ubiquitin-proteasome or lysosome-vacuolar compartments. Ubiquitin covalently links to the epsilon-amine of lysine (K) in target proteins and to one or more of seven K residues in protein-linked ubiquitin, forming branched polyubiquitin chains with different structural topologies capable of directing intracellular trafficking and fate of the tagged protein. In contrast to other receptor tyrosine kinases that are monoubiquitinated and targeted for lysosomal decay, ERBB2 is thought to be ubiquitinated with K-48 branched chains directing its proteasomal degradation. Immunblotting and confocal imaging have recently shown that when the proteasome is inhibited by PS341 (bortezomib) in ERBB2 overexpressing SKBR3 breast cancer cells, total and surface localized ERBB2 receptor decline within 12−24 h and ERBB2 accumulates in a perinuclear compartment having lysosomal characteristics (e.g. LAMP1/2-positive) and ubiquitin immunoreactivity. Polyubiquitinated ERBB2 appears in concert with gain of Hsp70 and loss of Hsp90 chaperone proteins co-associating with the receptor. Co-treatment of cells with lysosome inhibitors (chloroquine, CA-074-Me) has no effect on ERBB2 receptor trafficking but prevents PS341-induced decline in total ERBB2. To explore the role of K-specific polyubiquitination in targeting ERBB2 for proteasomal and/or lysosomal decay, we developed a multiple reaction monitoring (MRM) mass spectrometry (MS) procedure using a 4000 QTRAP to measure relative levels of endogenous K-48, K-63 and K-29 branched polyubiquitin linked to undegraded ERBB2 receptor immunoprecipitated from SKBR3 cells. MRM-MS analysis showed a rapid rise in K-48 ubiquitinated ERBB2 within 4 h of bortezomib treatment, reaching a 16.5-fold increase by 10 h. In a more delayed fashion, K-63 and K-29 ubiquitinated ERBB2 increased 40-fold and 10-fold, respectively, after 10 h of proteasome inhibition. These changes in receptor trafficking and polyubiquitin chain topology suggest that the accumulation of undegraded K-48 ubiquitinated ERBB2 triggers formation of K-63 and K-29 ubiquitinated ERBB2, redirecting receptor to an alternative degradation pathway within the lysosome.

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In vivo antitumor efficacy of TAK-285, a novel ErbB1/ErbB2 dual kinase inhibitor

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Background: HER2 and EGFR are promising targets for effective anticancer drugs. TAK-285, a novel HER2/EGFR dual kinase inhibitor is currently in clinical trials in the United States and Japan. In this study the in vivo efficacy of TAK-285 is demonstrated.
Methods: In vivo antitumor efficacy were examined using xenografts of BT-474 or 4−1ST, which express aberrant levels of HER2. A431, which overexpresses EGFR, was also used. TAK-285 was orally administered twice a day and efficacy was determined by: (growth volume of treated