

THE XIAP ANTISENSE COMPOUND AEG35156 / GEM640 DEMONSTRATES SIGNIFICANT ANTITUMOR ACTIVITY IN MULTIPLE HUMAN CANCER XENOGRAFT MODELS

2947



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XIAP is the most potent anti-apoptotic IAP. It inhibits two key effector caspases (caspases-3 & -7) and a key initiator caspase (caspase-9). Through inhibition of these caspases, XIAP effectively blocks apoptosis triggered by intrinsic (i.e. mitochondrial-related) as well as extrinsic (i.e. death receptor-related) insults. Its over-expression inhibits apoptosis arising from chemotherapy, ionizing radiation, growth factor deprivation, hypoxia, TRAIL, and other apoptotic triggers relevant to cancer. XIAP is over-expressed in all 60 members of the NCI cell line panel and is over-expressed or upregulated in many tumors. In collaboration with Hybridon Inc., Aegera has developed AEG35156/GEM640, a XIAP antisense molecule. This presentation summarizes the data from two different approaches aimed at down-regulating the expression of XIAP: RNAi and antisense oligonucleotides. Furthermore, the pre-clinical data demonstrating the effectiveness of AEG35156/GEM640 both as a single agent and particularly in combination with clinically relevant cytotoxic agents is shown.

Fig. 1: XIAP antisense is effective in reducing cellular XIAP protein levels both in vitro and in vivo

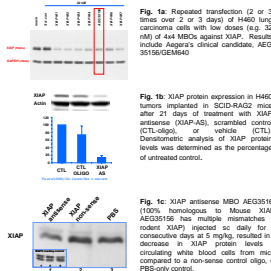


Fig. 2a: Anti-tumor effect of AEG35156 / GEM640 as a single agent in nude mice implanted with PC3 human prostate carcinoma cells.

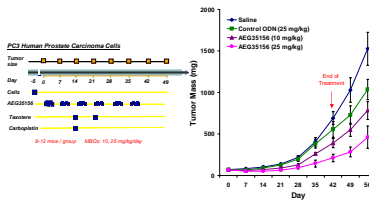


Fig. 2b: Anti-tumor effect of AEG35156 / GEM640 in combination with Taxotere or carboplatin in nude mice implanted with PC3 human prostate carcinoma cells.

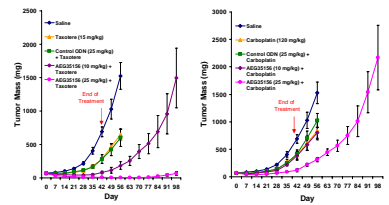


Fig. 3: Anti-tumor effect of AEG35156 / GEM640 in nude mice implanted with H460 human lung carcinoma cells.

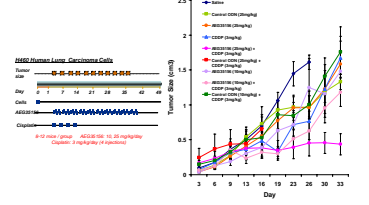


Fig. 4: Anti-tumor effect of AEG35156 / GEM640 as a single agent in nude mice implanted with LS1747 human colon carcinoma cells.

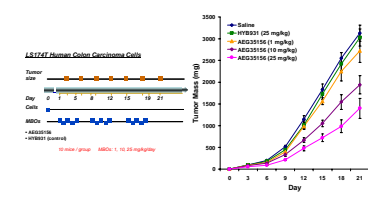
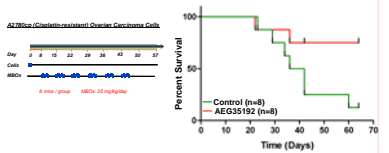


Fig. 5: Survival effects of XIAP antisense treatment in female nude mice implanted peritoneally with A2780cp human ovarian carcinoma cells.



Animal were treated daily i.p. with 25 mg/kg AEG35156, following a 5 days on, 2 days off, regimen for 42 days. Antisense treatment was initiated 8 days following cell implantation, a time in which necropsy results show multiple tumors to be established on the peritoneal lining. Results indicate that 6 of 8 animals survived greater than 70 days in the antisense treated group relative to 1 of 5 in the control group.

Fig. 6a: The stable RNAi-mediated loss of XIAP in MDA-MB-231 breast carcinoma cells leads to enhanced TRAIL-mediated caspase activation and processing of BID and PARP.

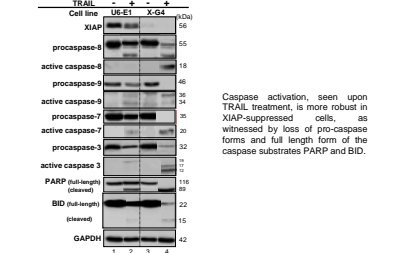
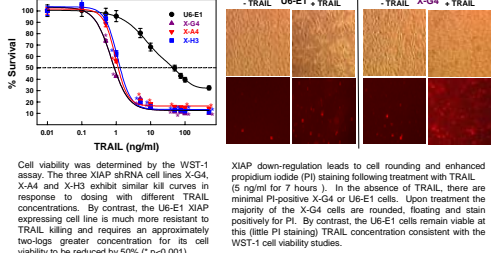
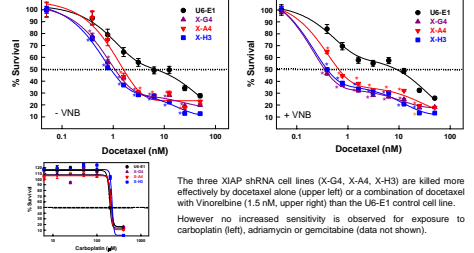


Fig. 6b: The stable RNAi-mediated loss of XIAP leads to enhanced sensitization of MDA-MB-231 cells to TRAIL killing.



Cell viability was determined by the WST-1 assay. The three XIAP shRNA cell lines X-G4, X-A4 and X-H3 exhibit similar cell curves in response to dosing with different TRAIL concentrations. By contrast, the UE-E1 XIAP expressing cell line is much more resistant to TRAIL killing and requires an approximately two-fold greater concentration for its cell viability to be reduced by 50% (*p<0.001).

Fig. 6c: The stable RNAi-mediated loss of XIAP in MDA-MB-231 breast carcinoma cells leads to enhanced sensitivity to Docetaxel alone or in combination with Vinorelbine.



XIAP down-regulation leads to cell rounding and enhanced propidium iodide (PI) staining following treatment with TRAIL. In the absence of TRAIL, there are minimal PI-positive X-G4 or UE-E1 cells. Upon treatment the majority of the X-G4 cells are rounded, floating and stain positively for PI. By contrast, the UE-E1 cells remain viable at this (little PI) staining TRAIL concentration consistent with the WST-1 cell viability studies.

Summary and clinical plans

- AEG35156 / GEM640 is effective in reducing tumor burden as a single agent in xenograft models of human prostate, lung, colon and ovarian cancer.
- Consistent with its mechanism of action, AEG35156 / GEM640 is most effective when combined with cytotoxic agents, such as Taxotere and carboplatin. Complete tumor regression was observed with AEG35156 / GEM640-Taxotere combination in established PC3 prostate xenografts.
- Pre-clinical toxicology studies in rodents and primates indicate AEG35156 / GEM640 to be well-tolerated (refer to poster # 2953).
- AEG35156 / GEM640 is scheduled to enter the clinic in March, 2004 in the United Kingdom as a single agent in an open-label, 2-center Phase 1 trial.
- RNA interference (RNAi) based approaches at suppressing XIAP expression provide a surrogate to AEG35156 for testing the effects of apoptosis-inducing agents in vitro.
- XIAP RNAi based stable breast cancer cell lines are equivalently sensitive to TRAIL and taxanes (Taxotere and Taxol), but not carboplatin, adriamycin or gemtabine (not all data shown).
- In conclusion the removal of the caspase inhibitor XIAP by antisense or RNAi approaches leads to improved responses to specific chemotherapeutic agents in vitro and in vivo.