

ANTITUMOR ACTIVITY OF XIAP ANTISENSE COMPOUND, AEG35156, CORRELATES WITH SUPPRESSION OF XIAP mRNA AND PROTEIN LEVELS IN HUMAN CANCER MODELS

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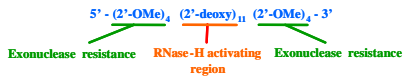
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Abstract and Introduction

The ability of cancer cells to evade apoptosis is a major factor in resistance to standard chemotherapies. Over-expression and/or deregulation of X-linked Inhibitor of Apoptosis (XIAP), a potent cellular inhibitor of caspases 3, 7 and 9, permits cancer cells to evade apoptosis. Blocking XIAP represents a promising strategy for cancer therapy. AEG35156 is a second-generation 2'-O-methyl-RNA/DNA antisense to XIAP, currently in clinical development, displaying potent cellular inhibition of XIAP expression. There is correlation between pharmacokinetic and pharmacodynamic effects of AEG35156 in animal models of cancer and in primates. In cultured human cancer cell lines, AEG35156, at 30-100 nM, reduced XIAP-RNA by >90% (measured by Taqman) and correspondingly reduced protein levels (measured by Western blot, flow cytometry and immunohistochemistry [IHC]). In pre-clinical development it was shown that AEG35156 reduced XIAP mRNA and protein levels in representative tissues at therapeutically feasible doses. A murine-specific variant of AEG35156 attenuated XIAP protein in circulating PBMCs and bone marrow when administered to mice subcutaneously at < 10 mg/kg. In a series of human tumor xenograft studies in mice, AEG35156, dosed at 10 and 25 mg/kg effectively suppressed XIAP protein levels in IHC sections of H460 human lung carcinoma. These doses achieved marked reductions in the rate of tumor growth, particularly when combined with suboptimal doses of docetaxel. A continuous IV infusion study in cynomolgus monkeys revealed that AEG35156 reduced XIAP protein levels in liver in a dose-dependent manner, as measured by Western and IHC analyses. XIAP liver protein levels fell 60% and 80% in animals infused with 2.5 and 10 mg/kg AEG35156, which were associated with steady state plasma levels of 0.250 and 1.25 µM, respectively. A Phase 1, 7 day continuous IV infusion evaluation of AEG35156, as a single agent, conducted in collaboration with the Cancer Research-UK has resulted in steady-state plasma levels of 0.2 to 0.6 µM. Pharmacokinetic and pharmacodynamic results will be updated on these studies. A Phase 1 trial with AEG35156, in combination with docetaxel, was recently initiated by the National Cancer Institute of Canada, and an AML trial, in combination with idarubicin and ara-C, commenced in the fall of 2005 in the US and Canada.

This presentation summarizes our data showing that AEG35156 is effective in reducing XIAP mRNA and protein levels in cancer cell lines (*in vitro*) and in xenograft models (*in vivo*) and that these changes are accompanied by anti-tumor activity (stand alone and synergistically with docetaxel). Furthermore, a continuous IV infusion study of clinically relevant doses of AEG35156 in cynomolgus monkeys revealed that XIAP mRNA and protein levels were reduced in a dose-dependent manner.



AEG35156 is a 19-mer 2nd generation mixed backbone oligonucleotide (MBO) with a fully phosphorothioated backbone consisting of 4 consecutive 2'-OMe-ribonucleotide residues at both the 3'- and 5'-ends and 2'-deoxyribonucleotide residues in the middle.

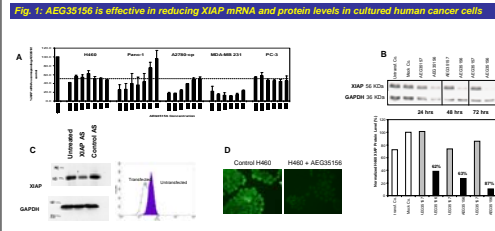


Fig. 1: AEG35156 is effective in reducing XIAP mRNA and protein levels in cultured human cancer cells
 A. Knockdown of XIAP mRNA *in vitro* in five human cancer cell lines transfected with increasing doses of AEG35156. Results expressed as percent XIAP mRNA levels for each dose antisense compared to the corresponding dose of control oligonucleotide (AEG35185). B. XIAP protein knockdown *in vitro* with transfected H460 non-small-cell lung carcinoma cells which were repeatedly transfected for 1, 2 or 3 days with 31 nM of AEG35156 or control oligonucleotide (AEG35187). Percent knockdown of XIAP protein was calculated based on GAPDH-normalized densitometric readings and reported relative to control oligonucleotide values. C. Knockdown of XIAP protein in Jurkat cells treated *in vitro* with XIAP antisense. Cells were cultured in the presence of unopsonized Zsolt XIAP antisense for 3 days. XIAP levels were detected either by western blot or by flow cytometry using a FITC-conjugated anti-XIAP monoclonal antibody. D. Knockdown of XIAP protein in cultured H460 cells treated with AEG35156. Cells transfected with AEG35156 (48hrs) and then stained for XIAP using a XIAP specific monoclonal (BD48) followed by HRP-Tyramide Alexa 488.
Results: AEG35156 potently knocks-down XIAP mRNA *in vitro* in a variety of cancer cell lines with an EC50 of 0-32 nM and this is accompanied by a 60-87% reduction in XIAP protein.

Fig. 2: AEG35156 suppresses XIAP protein levels in a H460 human lung carcinoma xenograft model in mice and reduces tumor growth either alone or in combination with Taxotere

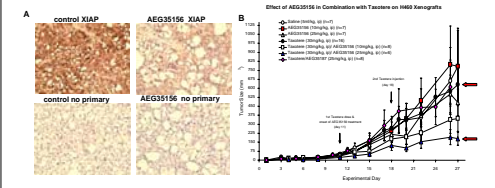


Fig. 2: AEG35156 suppresses XIAP protein levels in a H460 human lung carcinoma xenograft model in mice and reduces tumor growth either alone or in combination with Taxotere
 A. XIAP protein levels are reduced in a tumor xenograft model after treatment with AEG35156, as demonstrated by IHC. Animals bearing H460 xenograft tumors were treated with AEG35156 (25 mg/kg, ip) for 8 days *in vivo*. Tumors were excised, cryoprotected and stained with anti-XIAP, amplified with HRP-Tyramide, and then revealed with streptavidin-HRP and DAB. B. AEG35156 has anti-tumor effects when given alone or in combination with Taxotere in nude mice implanted with H460 human lung carcinoma cells. Animals were treated daily i.p. with 10 or 25 mg/kg AEG35156, or with 25 mg/kg control MBO (AEG35187), following a 5 days on, 2 days off regimen. Treatment with antisense was initiated 11 days after cell implantation when tumors were ~50-70mm³. Taxotere (30 mg/kg, ip) was co-administered to the indicated groups on days 11 and 18.
Results: AEG35156 caused a dose-dependent regression of established tumors particularly when administered in combination with Taxotere (red arrows). These anti-tumor results correlate with an *in situ* reduction in XIAP protein.

Fig. 3: A murine-specific variant of AEG35156 attenuates XIAP protein in bone marrow and circulating PBMCs when administered to mice subcutaneously

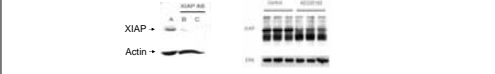


Fig. 3: A murine-specific variant of AEG35156 attenuates XIAP protein in bone marrow and circulating PBMCs when administered to mice subcutaneously
 Left panel: CD1c mice were treated with 25 mg/kg AEG35169 XIAP antisense (100% homologous to mouse XIAP) ip for 3 weeks. Bone marrow was isolated and XIAP expression determined by Western blot (A, control animal; B, C: XIAP-AS treated animals). Right Panel: Antisense treatment reduces XIAP in murine lymphocytes. Mice treated with AEG35169 10 mg/kg ip daily for 3 weeks.
Results: XIAP antisense attenuated XIAP protein levels in PBMCs and bone marrow.

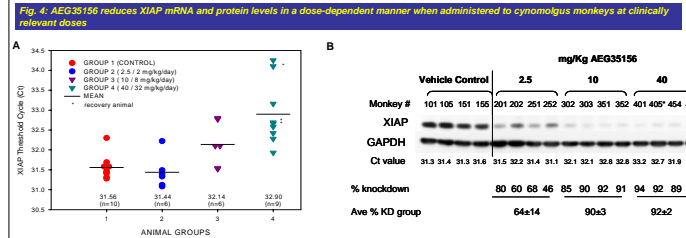


Fig. 4: AEG35156 reduces XIAP mRNA and protein levels in a dose-dependent manner when administered to cynomolgus monkeys at clinically relevant doses
 A. XIAP mRNA knockdown in normal cynomolgus monkey liver after treatment with AEG35156 (one top difference with monkey XIAP target area), as judged by TaqMan cycle threshold (Ct) values. Treatment regime was by continuous IV infusion for two 7-day cycles (cycle 1: 2.5, 10 or 40 mg/kg/day, cycle 2: 2, 8 or 32 mg/kg/day, respectively) with a 2 week treatment-free period between cycles. Recovery animals had a further 2 week drug-free period after completion of the second dose period (individuals denoted by *). Mean Ct values are given for each test group along with the number (n) of individuals in the group. XIAP Ct values were normalized to GAPDH.
Results: AEG35156 caused a dose-dependent reduction in XIAP-mRNA, up to 83%, in monkey liver tissue after treatment with > 10 mg/kg/day AEG35156. Reduction in XIAP message was still evident 2 weeks post-treatment in the recovery animals.
 Western blot analyses of XIAP protein in cynomolgus monkey liver samples after treatment with AEG35156. TCA-precipitated cytosolic fractions of liver samples are presented. Percent knockdown (KD) was determined by densitometric analysis and represents XIAP values, normalized to GAPDH, relative to normalized control values. KD for male animals (designated with ♂) and female (♀) animals correspond to a subset of those studied in Figure 4A. Recovery animals (*).
Results: AEG35156 induced a dose-dependent reduction in cytosolic XIAP protein levels in monkey liver, with up to 92% loss of XIAP at the high dose. XIAP protein suppression was also evident in recovery animals (14 days after their last AEG35156 treatment), demonstrating long-term antisense effects.

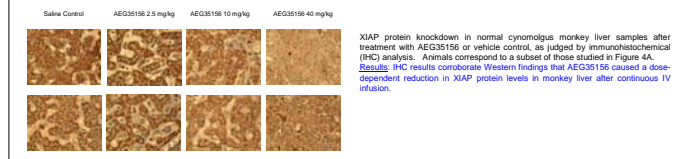


Fig. 5: Steady-state plasma levels of AEG35156 (and its major metabolites) correlate between cynomolgus monkeys and humans at escalating doses of continuous IV infusion

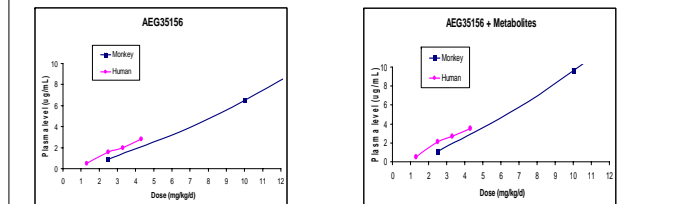


Fig. 5: Steady-state plasma levels of AEG35156 (and its major metabolites) correlate between cynomolgus monkeys and humans at escalating doses of continuous IV infusion
 Venous blood samples obtained at 3, 6, 24, 72 and 168 hrs post start of continuous IV infusion were analyzed at CTRB (Montreal) for plasma levels of AEG35156 and its major metabolites (N-1, N-2) using capillary gel electrophoresis (CGE).
Results: Steady-state plasma concentrations of AEG35156 and its major metabolites achieved rapidly (6 hrs) after the start of infusion, were approximately dose-proportional and correlated closely between monkeys and humans; significant formation (> 30% of total oligonucleotides) of metabolites occurred in both monkeys and humans.

Conclusions

- The XIAP antisense compound, AEG35156, suppressed XIAP-RNA and protein levels in cultured cells at low nanomolar concentrations [Figure 1]
- AEG35156 reduced XIAP protein levels in human xenograft tumor models, under conditions where tumor growth was effectively suppressed [Figure 2]
- XIAP antisense reduced XIAP levels in circulating PBMCs in mice, supporting the use of these cell populations as a possible surrogate for XIAP knockdown in target tissues in the clinical setting [Figure 3]
- At doses shown to be clinically acceptable (i.e.; 2.5 to 10 mg/kg/day), AEG35156 caused a dose-dependent suppression of XIAP protein in the liver of cynomolgus monkeys [Figure 4], and other tissues

AEG35156 is currently being evaluated in three clinical trials:

- Phase 1 single agent in refractory solid tumors (UK)
- Phase 1 combination trial with docetaxel in docetaxel-sensitive tumors (Canada)
- Phase I/II combination trial with araC/darubicin in refractory/relapsed AML (US & Canada)

Note: This preclinical data is in support of PK and PD studies that form part of the preliminary Phase 1 clinical study results of AEG35156, presented by Dr. M. Ranson (Poster C72, Thursday)