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ORAL

AT13387, a Heat Shock Protein 90 Inhibitor in a Phase I Study Exhibits Potent Activity in GIST Models

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Background: AT13387 is a highly potent Heat Shock Protein 90 (HSP90) inhibitor (IC₅₀ 0.7 nM) derived from Astex' novel fragment chemistry platform, PyramidTM. Preclinical experiments show that AT13387 suppresses client proteins for greater than 7 days in tumour cells, making it the longest acting agent reported to date. 17-AAG, an HSP90 inhibitor is a first generation natural product from the ansamycin class and it has exhibited early activity in clinical trials against GIST tumours. Most gastrointestinal stromal tumours (GISTs) express constitutively activated mutant isoforms of KIT or kinase platelet-derived growth factor receptor alpha (PDGFRA) that are potential therapeutic targets of kinase inhibitors. After initial response to therapy, most patients fail treatment and become refractory to sequential treatment with kinase inhibitors due to the emergence of new mutations. Since mutant forms of KIT are clients of HSP90, we reasoned that AT13387 could sensitize GIST tumour cell lines. Moreover, GIST cell lines acquire resistance to first generation inhibitors such as 17-AAG through loss of NAD(P)H: quinine oxidoreductase 1.

Methods: Four cell lines were tested for sensitivity to AT13387 in vitro. The cell lines, GIST 882 and T1 are cell lines which express activating KIT mutation in exons 13 and 11 respectively, GIST48 and GIST430 are human cell lines established from GISTs progressing on imatinib therapy and GIST 430B is a 17-AAG resistant sub-line. GIST48 has a homozygous *KIT* exon11 mutation (V560D) and a heterozygous *KIT* exon17 mutation (D820A). GIST430 has heterozygous mutations in *KIT* exon11 (in-frame deletion) and *KIT* exon13 (V654A). AT13387 was compared to 17-AAG, imatinib and sunitinib in a number of in vitro assays. GIST 48B represents a KIT independent cell line derived from a GIST patient. Proliferation assays tested the relative sensitivity of each mutation to each compound. Subsequent western blotting of cleaved PARP as well as analysis of both the pERK pathway and the PI3 kinase pathways were compared on representative cell lines.

Results: AT13387 displayed impressive activity against a panel of GIST cell lines. The HSP90 inhibitor was as active or more active than 17-AAG in all the cell lines tested especially the GIST 430B (1mM versus 75nM). AT13387 was also active in imatinib and sunitinib resistant GIST cell line (380nM versus >1 mM for imatinib and sunitinib). AT13387 was more active on imatinib resistant cell line than sunitinib (100nM versus 285nM).

Conclusions: AT13387 is in Phase I clinical trials for advanced solid tumours and based on the pre-clinical work presented warrants further study in an imatinib refractory GIST population.

Poster Presentations (Mon, 26 Sep, 09:30–12:00)

Sarcoma: Soft Tissue and Bone

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POSTER

Targeting VEGF in Human Angiosarcoma Cell Lines in Vitro

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Background: As malignant tumours of endothelial cells there is intense interest in the potential of anti-angiogenic therapy for angiosarcomas. We have performed a series of *in vitro* studies to investigate the role of angiogenesis in two human cutaneous angiosarcoma cell lines (ASM and ISO-HAS), using human dermal microvascular endothelial cells (HuDMECs) as controls.

Methods: A protein array study (R&D Systems) was used to assess the relative expression of angiogenic proteins in cell lysates. The concentration of vascular endothelial growth factor (VEGF) in supernatants was quantified by ELISA. Western blotting (WB) was used to investigate the expression of VEGF isoforms in supernatants and VEGFR2 status in cell lysates. The effects of axitinib (A) a VEGFR tyrosine kinase inhibitor and bevacizumab (Bz) the humanised monoclonal antibody to VEGF were investigated *in vitro*. Functional studies were performed including proliferation assay, tubule formation assay on Matrigel and migration studies using the Boyden chamber.

Results: The protein array identified increased expression of angiogenin, aFGF, bFGF, HGF and VEGF in the malignant cell lysates relative to HuDMECs. VEGF was increased 6.3 fold in ASM (p=0.027) and 4.3 fold in ISO-HAS (p=0.073). The ELISA showed significantly elevated levels of VEGF in the supernatant of ASM and ISOHAS relative to HuDMECs

(mean VEGF HuDMECs 24±9 pg/ml vs ASM 180±58 (p=0.038) vs ISO-HAS 150±58 (p=0.043)). WB of supernatant identified a predominant VEGF band at 20 kDa consistent with VEGFA₁₆₅ monomer. WB for pVEGFR2 suggested no autocrine or paracrine stimulation in the cell lines. Proliferation assays identified no significant effect of A or Bz as single agent therapy. Results of other functional assays are shown in the table, with both agents significantly reducing tubule formation in ISO-HAS, but only Bz in the ASM cell-line.

	Change (%)					
	Axitinib			Bevacizumab		
	Proliferation Viable cell count	Matrigel Number of tubules	Migration Cell count	Proliferation Viable cell count	Matrigel Number of tubules	Migration Cell count
ASM	-9% P=0.314	-5% p=0.791	-9% p=0.458	18% P=0.162	-46% p=0.003	-10% p=0.113
ISO-HAS	0% p=0.846	-37% p=0.018	-14% p=0.244	-7% P=0.295	-30% P=0.006	-23% p=0.144

Conclusion: The protein studies support the rationale of targeting VEGF in angiosarcoma. However the effects of VEGF targeted therapy as a single agent *in vitro* were modest. Further investigation of the role of VEGF signalling in angiosarcoma biology is required.

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POSTER

Mitochondrial Proliferation Induces Apoptosis in Human MFH Cells by PGC-1alpha Overexpression

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Introduction: High-grade sarcomas, such as malignant fibrous histiocytoma (MFH), are clinically aggressive and have a high metastatic behavior. The prognosis of patients can be poor due to local recurrence and metastases, so new therapeutic strategies against sarcomas are eager to be established. Previous reports suggest that a decrease of mitochondrial number may lead to cancer development, and that stimulation of mitochondrial activity may be an efficient tool in anticancer strategy. Peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1α) is a multi-functional transcription coactivator that regulates the mitochondrial biogenesis through the mitochondrial transcription factor A (TFAM).

The purposes of this study were to verify the expression of mitochondrial DNA (mtDNA), PGC-1α and TFAM in musculoskeletal tumours, and to examine the effect of PGC-1α overexpression on apoptosis of human MFH cells.

Methods: We used 39 musculoskeletal tumour samples including 27 malignant tumours and 12 benign tumours, and human MFH cell line, Nara-H. We evaluated the relative expression of the mtDNA content to the nuclear DNA, and the expression of PGC-1α and TFAM in 39 samples by quantitative real-time PCR. To investigate how PGC-1α and mitochondria affected cell apoptosis, PGC-1α overexpressing plasmid (PGC-1α plasmid) was transfected into Nara-H cells with or without TFAM-siRNA, and we analyzed for DNA fragmentation and mitochondria-selective probes by flow cytometry and fluorescent immunostaining. To verify if the mitochondrial involvement in this type of apoptosis, we performed immunoblotting for the expression of apoptosis related proteins.

Results: In musculoskeletal tumour samples, we found that the mtDNA in malignant tumours was statistically lower than that in benign tumours, and that the decrease of relative mtDNA was accompanied by the decrease of PGC-1α and TFAM.

Flow cytometry and fluorescent immunostaining showed that PGC-1α overexpression induced MFH cell apoptosis with an increase of mitochondrial proliferation. However, both the induction of apoptosis and the increase of mitochondrial proliferation were not found in TFAM-siRNA transfected cells. In PGC-1α overexpressing cells, the expression of cleaved caspase 3 and 9, and cytochrome C, but not cleaved caspase 8 was increased compared with control.

Discussion: It has not been reported that mitochondrial proliferation induced tumour cell apoptosis. However, in this study, we found that the decreases of mtDNA, PGC-1α and TFAM expression were detected in malignant musculoskeletal tumours, and that mitochondrial proliferation by PGC-1α overexpression induced human MFH cell apoptosis. Our findings suggest that PGC-1α dependent mitochondrial biogenesis might be involved in MFH cell growth and may be a potent therapeutic target for human MFH.