Astex presents updates on AT7519 and AT9283 at the American Society of Hematology (ASH) Conference

Cambridge, UK, 1st December 2008

Astex Therapeutics Limited, the leading fragment based drug discovery company, today announced that it is to present key findings on two of its clinical stage compounds, AT7519 and AT9283, at the 50th American Society of Hematology (ASH) Annual Meeting and Exposition, 6-9th December, 2008, San Francisco, CA, USA.

Loredana Santo MD, representing a group of collaborating scientists from the Dana Farber Cancer Institute, Massachusetts General Hospital and the University of Turin, will make an oral presentation describing pre-clinical data on Astex’s cyclin dependent kinase (CDK) inhibitor AT7519 showing significant anti-tumour activity in models of multiple myeloma (MM). Data will also be presented by collaborating scientists from the Arizona Cancer Center showing that AT7519 has a cytotoxic effect on tumour samples from patients with chronic lymphocytic leukaemia (CLL). Together, these data provide the rationale for further clinical evaluation of AT7519 in the treatment of multiple myeloma and CLL and suggests that AT7519 also offers a promising potential treatment strategy for patients with advanced B-cell leukemia and lymphoma. AT7519 has now completed one Phase I trial in patients with solid tumours and is being tested using an alternative dosing schedule in a second Phase I trial in patients with solid tumours.

New data from further pre-clinical studies on its multi-targeted kinase inhibitor, AT9283, currently in Phase I/IIa in patients with advanced haematological malignancies, will also be presented by collaborating scientists from Kyoto University Hospital and the University of Fukui. The data show that AT9283 has potent anti-proliferative activity in a panel of human cell lines expressing drug-resistant mutant forms of BCR-ABL as well as increased survival rates in vivo model systems following multiple cycles of treatment with AT9283. These data together support further clinical investigation of AT9283 in patients with treatment resistant chronic myeloid leukaemia (CML).

Data were presented at ASH in 2007 from the current Phase I/IIa clinical study of AT9283 in patients with haematological malignancies indicating that the compound is active in the treatment of a proportion of patients with relapsed/refractory acute myeloid leukaemia (AML). On this occasion we report studies designed to help enrich patient populations for those patients that may be sensitive to treatment with AT9283. Using AML cell lines from a variety of genetic backgrounds, including a proportion driven by ras, Flt3 or c-kit mutations, Astex has generated preliminary pre-clinical data that indicate that patients with mutations in oncogenic signalling pathways may be particularly sensitive to treatment with AT9283. This hypothesis is currently being explored through the analysis of biological samples obtained from the ongoing clinical program.

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Editor's Notes

i) Oral Presentation Title: AT7519, a Novel Small Molecule Multi-Cyclin Dependent Kinase Inhibitor, Induces Apoptosis in Multiple Myeloma Via GSK3β

Session Date: Monday December 8, 2008: 8:30 AM
Session ID: Pathophysiology and Translational Models
Session Location: Moscone Center 2009-2011-2022-2024-West
Presenter: Loredana Santo MD, Dana Farber Cancer Institute
Abstract Number: 251

ii) Poster Title: AT7519, a Potent Multi-Targeted CDK Inhibitor, Is Active in CLL Patient Samples Independent of Stage

Session Date: Monday, December 8, 2008: 5:30 PM
Session ID: Chronic Lymphocytic Leukemia - Therapy, Excluding Transplantation Poster II
Session Location: Hall A (Moscone Center)
Abstract Number: 3161
Poster Board: III-243
Cyclin dependent kinases (CDKs) and their cyclin complexes play a crucial role in cell cycle control and transcriptional regulation. In multiple myeloma (MM), the abnormal activation of different CDKs and their cyclin partners, especially CDK4/cyclin D1 and CDK6/Cyclin D2, mediate uncontrolled cell cycle progression. Therefore CDKs represent promising novel therapeutic targets for MM. Additionally the cytokine dependent PI3K/Akt signaling pathway mediates growth, survival, drug resistance, migration and cell cycle regulation in MM. Activated Akt in turn phosphorylates downstream target molecules like glycogen synthase kinase (GSK)-3β impacting growth and survival. Here we investigated the preclinical activity of a novel small-molecule multi-CDK inhibitor, AT7519 in MM. In vitro kinase assays demonstrated more potent inhibition of CDK 1, 2, 4, 5 and 9 compared to CDK 3, 6, and 7. AT7519 also demonstrated potent inhibitory activity against GSK-3β. No significant inhibitory effects against other kinases were observed. We next investigated the growth inhibitory effect of AT7519 on MM cell lines. Maximal cytotoxicity was observed in 48 hour culture with IC50 values ranging from 0.5μM (MM.1S, U266) to 4 μM (MM1R). AT7519 was also effective against primary tumor cells from MM patients with no significant cytotoxicity noted in peripheral blood mononuclear cells from healthy volunteers. To delineate the underlying mechanism of cytotoxicity induced by AT7519, cell cycle analysis using PI staining in MM.1S cell line was performed. No significant accumulation of cells in a particular phase of cell cycle was noted; however, AT7519 showed an increased sub-G1 population, indicative of apoptosis, which was confirmed by Annexin V+PI+ staining and associated with caspase-8-9 and -3 cleavage. Importantly, we found that AT7519 markedly inhibited phosphorylation (serine 2 and serine 5 sites) of the carboxyl terminal domain of RNA polymerase II (RNA pol II) within 6 hours of treatment. Non-cell cycle CDKs including CDK9 are responsible for phosphorylation and activation of RNA pol II. Similarly, AT7519 also inhibited phosphorylation of GSK-3β while no significant effects on CDK expression levels were evident at early time points. To investigate whether there was a correlation between inhibition of phosphorylation of GSK-3β and RNA pol II, MM.1S cells were cultured with a-amanitin, a specific inhibitor of RNA pol II. Although phosphorylation of RNA pol II was significantly inhibited, phosphorylation of GSK-3β was not altered by a-amanitin (10 μM for up to 24 hours). These results suggest that GSK-3β and RNA pol II dephosphorylation at serine 2 and serine 5 may be two independent mechanisms by which AT7519 induces apoptosis in MM cells. Ongoing studies are confirming the role of GSK-3β in AT7519 induced cytotoxicity of MM cells. Finally, the in vivo efficacy of AT7519 was examined using a xenograft mouse model of human MM. Mice treated with AT7519 demonstrated slower tumor growth compared to the control group without adverse effects. Moreover, AT7519 resulted in a significant prolongation in median overall survival in treated mice (40 days in the treatment group versus 27.5 days in the control cohort, p = 0.0324). In conclusion, these results show significant anti-MM activity of AT7519, and provide the rationale for its clinical evaluation in MM.

Cyclin Dependent Kinases (CDKs) play a central role in the eukaryotic cell cycle. The activation of these kinases is modulated by the expression and binding of their regulatory cyclin partners. Their key role in cell cycle progression, coupled to evidence that pathways leading to their activation are deregulated in a number of human cancers makes them attractive therapeutic targets. More recently the role of CDKs 7, 8 and 9 in the regulation of transcription has been explored. CDK9 has been shown to play a role in the regulation of transcription via phosphorylation of RNA polymerase II (RNA pol II). The outcome of transcriptional inhibition via CDK9 exhibits significant variation between cell lines. B-Cell lymphoproliferative disorders, including CLL, rely on the expression of transcripts with a short half-life such as Mcl-1, Bcl-2 and XIAP for survival. In vitro studies have demonstrated that compounds with transcriptional inhibitory effects are effective pro-apoptotic agents in models of this disease.
AT7519 was shown to induce apoptosis (by MTS, morphology and PARP cleavage) in these samples at concentrations of 100-700nM. AT7519 appears equally effective at inhibiting the survival of CLL cells harbouring a variety of mutations including those representative of patients that fall within poorer prognosis treatment groups. The amount of AT7519 required to induce cell death in 50% of the CLL cell population increased as exposure time was decreased but significant cell death was obtained at doses approximating to 1uM following 4-6h of treatment. These doses are equivalent to exposures achieved in ongoing AT7519 clinical studies indicating that cytotoxic doses can be achieved in patients on well tolerated schedules.

The mechanism of AT7519 cytotoxic effects was investigated by western blotting for a variety of cell cycle and apoptotic markers following incubation with compound. Short term treatments (4-6h) resulted in inhibition of phosphorylation of the transcriptional marker RNA pol II and the downregulation of the anti-apoptotic protein Mcl-1. Additional anti-apoptotic proteins including XIAP and Bcl-2 remained unchanged. The reduction in Mcl-1 protein levels was associated with an increase in the apoptotic marker cleaved PARP.

No inhibition of cell cycle markers such as phospho-retinoblastoma protein was observed in the same samples suggesting that the cytotoxic effects of AT7519 in CLL patient samples is due to its transcriptional activity alone.

Together the data suggest AT7519 offers a promising treatment strategy for patients with advanced B-cell leukemia and lymphoma.

iii) Activity of the Multi-Targeted Kinase Inhibitor, AT9283 on Imatinib-Resistant CML Models

Abstract Number: 1104

CML is caused by a consistent genetic abnormality, termed the Philadelphia chromosome, that results from a reciprocal (9;22) translocation leading to the expression of the BCR-ABL fusion protein. Although treatment has been revolutionized by the introduction of tyrosine kinase inhibitors which target Abl activity, reactivation of Abl signaling via several different point mutations remains problematic. In particular the mutation of Threonine 315 to Isoleucine (T315I) confers resistance to all existing therapies with tyrosine kinase inhibitors in the clinical settings. We describe the in vitro and in vivo effects of AT9283, a potent inhibitor of several protein kinases, including Abl kinase (wild type BCR-ABL and several of the drug resistant mutant variants that have arisen in clinical practice e.g. T315I), JAK2, JAK3 and Aurora kinases A and B, on imatinib-resistant CML cells including those harboring BCR-ABL (T315I). AT9283 has potent anti-proliferative activity in a panel of BaF3 and human cell lines expressing the BCR-ABL or its mutant forms. In BaF3 BCR-ABL wild-type and T315I mutant cells and K562 CML cells we observed inhibition of substrates of both BCR-ABL (STAT5) and Aurora B (Histone H3) at concentrations >300nM and <100nM, respectively, suggesting that AT9283 is capable of inhibiting Aurora and BCR-ABL simultaneously in these cell lines. The in vivo effects of AT9283 were examined in several mouse models engrafed either subcutaneously or intravenously with BaF3, human CML cell lines or primary CML patient samples expressing the BCR-ABL or its mutant forms. Specifically AT9283 prolonged the survival of mice engrafted intravenously with either BaF3 BCR-ABL T315I, or E255K cells when administered intraperitoneally twice daily at doses of either 6.25 or 10mg/kg or once daily at 15mg/kg when administered 5 days in every week repeated twice. Maximal survival advantage was conferred at either 10mg/kg twice daily or 15mg/kg once a day. Similar data were obtained in an intravenous model using primary CML cells taken from a patient harbouring the BCR-ABL E255K mutation. We also present data from ongoing studies showing increased survival rates in these in vivo model systems following multiple cycles of AT9283 administered on the 15mg/kg once daily schedule. These data together support further clinical investigation of AT9283 in patients with treatment resistant CML.

iv) Outcome of Aurora Kinase Inhibition of Acute Myeloid Leukemia by AT9283 Is Dependent upon the Presence or Absence of Mutations in Type 1 Oncogenic Kinase Signalling Pathways

Abstract Number 1613

Recent reports suggest that Aurora kinases (AK) A and B are overexpressed in a proportion of patients with AML and that the level of overexpression correlates with their sensitivity to AK inhibition in vitro. Inhibition of AKB results in mitotic exit in the absence of cell division resulting in polyploidy whilst AKA is responsible for the fidelity of mitotic spindle assembly and phosphorylates p53 at Ser315 leading to its ubiquitination by Mdm2 and subsequent proteolysis. Inhibition of this process increases p53 stability inducing cell-cycle arrest with 4N DNA. Clinical studies of the dual AK inhibitor AT9283 indicate that it is active in the treatment of a proportion of patients with relapsed/refractory AML. In order to explore the molecular basis of this varied sensitivity we investigated the effect of AT9283 in a panel of 10 AML cell lines from a variety of genetic backgrounds.
including a proportion driven by ras, Flt3 or c-kit mutations. Two phenotypes were observed; 1) Accumulation of cells in the G2/M phase (4N) of the cell cycle followed by apoptosis, or 2) Accumulation of cells with >4N DNA (polyploid) followed by apoptosis. Cell lines that exhibit the former phenotype were those driven by mutation in an oncogenic kinase such as ras, c-kit or Flt3. Further analysis of Cyclin B levels suggest that profile 1) results from a G2 block occurring as a consequence of a dominant AKA inhibitory effect in these cell lines. Profile 2) results from AKB inhibition; where cells continue to undergo rounds of DNA replication in the absence of cell division. Inhibition of both of these signaling pathways has been confirmed in AML blasts taken from patients treated with AT9283 in the ongoing clinical program. These results suggest that AT9283 triggers the mitotic checkpoint and induces apoptosis in patients harbouring mutations in FLT3 or c-kit via AKA inhibition. These AML cases have been shown to be more likely to exhibit normal cytogenetics and this profile may be important in sustaining rapid peripheral blast proliferation typical of this subtype of AML. Cell lines that respond to treatment with AT9283 by becoming polyploid may be manifesting the effect of predominant AKB inhibition. The balance between these outcomes may reflect the corresponding levels of expression of AKA and AKB in individual subtypes of AML along with factors such as TP53 mutation being associated with genomic instability supporting the development of complex karyotypic abnormalities. Such preliminary findings indicate that patients with mutations in oncogenic signaling pathways may be particularly sensitive to treatment with AT9283 due to the presence of an intact mitotic checkpoint and a dominant Aurora A inhibitory phenotype. This hypothesis is currently being explored through the analysis of biological studies obtained from the ongoing clinical program.

Astex Therapeutics

Astex is a UK-based biotechnology company that discovers and develops novel small molecule therapeutics. Using its pioneering fragment-based drug discovery platform Pyramid™, Astex has built a pipeline of five molecularly-targeted oncology drugs, of which three are currently being tested in clinical trials and two are in pre-clinical development.

In addition to its proprietary research programmes, Astex’s productivity in lead discovery has been endorsed through numerous partnerships with major pharmaceutical companies, including AstraZeneca, Bayer-Schering, Boehringer Ingelheim, Novartis and Johnson and Johnson.

For further information on Astex please visit the Company’s website at www.astex-therapeutics.com