Encouraging Data on Astex’s Anti-Cancer Drug AT9283 to be Presented at ASH

New Data Suggest a Potential Role for AT9283 in Combination With Lenalidomide and With Docetaxel in the Treatment of Multiple Myeloma and Non-Hodgkin’s Lymphoma Cambridge UK, 1st December 2010

Astex Therapeutics, the UK-based biotechnology company developing targeted therapies for oncology and virology, today announced presentations of new data on its combinatorial oncogenic kinase inhibitor AT9283. The presentations will be made during the 52nd American Society of Hematology Meeting, 4-7th December in Orlando.

AT9283 in Combination with Lenalidomide Results in Synergistic Anti-Myeloma Activity

Researchers from the Massachusetts General Hospital Cancer Center, Harvard Medical School and the Dana-Farber Cancer Institute, in Boston, MA, have evaluated the activity of Astex’s multi-targeted kinase inhibitor, AT9283, in combination with established multiple myeloma drugs and found a strong synergistic effect when AT9283 was combined with lenalidomide. The synergistic effect is thought to be due to the fact that the two drugs target different pathways and different phases of the cell cycle, thus augmenting their individual anti-myeloma activities. The results of the study provide a rationale for the clinical evaluation of AT9283 in combination with lenalidomide in multiple myeloma patients.

AT9283 Suppresses Tumor Growth in Aggressive B-Cell Non-Hodgkin’s Lymphoma

Researchers from the Arizona Cancer Center will present data from a study to examine whether targeting inhibition of mitosis with AT9283 would be effective in promoting apoptosis in aggressive B-Cell Non Hodgkin’s Lymphoma cell lines. The study demonstrated that the combination of AT9283 plus docetaxel resulted in a statistically significant level of tumor growth inhibition over controls at well tolerated doses. These results suggest that AT9283 plus docetaxel may represent a novel therapeutic strategy for patients with aggressive B-Cell Non Hodgkin’s Lymphoma.

AT9283 Biomarker Strategy in Paediatric Patients

This presentation will focus on the development of a biomarker to support the forthcoming Cancer Research UK Drug Development Office-sponsored Phase I trial for AT9283 in paediatric and adolescent patients with leukaemia. By adapting and validating a Plasma Inhibitory Activity (PIA) assay to detect inhibition of Aurora, ABL and FLT3 kinases, a method for the non-invasive assessment of the pharmacodynamic action of AT9283 has been developed that can easily be adapted to paediatric patients for whom limited volumes of blood are available for such studies. The PIA assay, which may help delineate important mechanisms of action for novel anti-leukaemic drugs, will be used to assess target kinase modulation in a Phase I trial of AT9283 in children and adolescents with relapsed and refractory acute leukaemia. This work is being conducted by a team at The Institute of Cancer Research in Sutton, UK, with funding from Cancer Research UK.

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About 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 molecularly targeted oncology drugs, of which three are currently being tested in clinical trials with a number of others 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, GlaxoSmithKline, Novartis and Johnson & Johnson.

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

Editors Notes: ASH presentations
Abstract Number: 2994

Presentation Title: AT9283, a Small Molecule Multi-Targeted Kinase Inhibitor with Potent Activity Against Aurora Kinase and STAT3 In Combination with Lenalidomide Results In Synergistic Anti-Myeloma Activity

Presentation Time: Sunday, December 5, 2010: 6:00 PM-8:00 PM
Poster Session: Myeloma - Pathophysiology and Pre-Clinical Studies, excluding Therapy: Poster I
Poster Venue: Hall A3/A4 (Orange County Convention Center)
Poster Board Number: II-874

Author Block:

Loredana Santo, MD, Teru Hideshima, MD, PhD, Diana Cirstea, MD, Madhavi L Bandi, PhD, Erik A Nelson, PhD, Hiroshi Ikeda, MD, Sonia Vallet, MD, Samantha Pozzi, MD, Kishan Patel, Gullu Gorgun, PhD, Naoya Mimura, MD, Claire Fabre, MD, Yiguo Hu, PhD, Dharminder Chauhan, PhD, Matthew S Squires, PhD, Nikhil C. Munshi, MD, Kenneth C. Anderson, MD and Noopur Raje, MD

1 MGH Cancer Center, Massachusetts General Hospital, Harvard Medical School, Boston, MA
2 The Jerome Lipper Multiple Myeloma Center, Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA
3 Astex Therapeutics Ltd., Cambridge, United Kingdom

Abstract Body:

Despite recent advances with new drugs such as bortezomib, thalidomide and lenalidomide, multiple myeloma (MM) remains an incurable disease. Used as single agents, these compounds have shown marked antitumor activity, but the number of patients with relapsed and refractory disease remains high. Combination of these agents with other classes of novel drugs would offer great promise to improve patient outcome. AT9283 (Astex therapeutics, Cambridge UK) is a multi-targeted kinase inhibitor that inhibits Aurora A (AURKA), Aurora B (AURKB) and Janus Kinase (JAKs). AURKA and AURKB expression has been correlated with genetic instability and cellular proliferation in MM; therefore, Aurora kinases represent an attractive therapeutic target in MM. In addition the JAK/STAT pathway plays an important role in the survival and proliferation of MM cells. Blocking this pathway may therefore be critical for the survival of MM cells. AT9283 decreased both phospho-Histone H3 and the phosphorylation of Aurora A at Thr 288 in Nocodazole treated cells, suggesting the dual activity of AT9283 against AURKA and AURKB. Importantly, besides Aurora kinase inhibition, we observed that AT9283 inhibited STAT3 tyrosine phosphorylation within 30 minutes of treatment. The effect of AT9283 on pSTAT3 inhibition was further investigated by using U3A cells stably expressing a luciferase reporter gene under the control of a STAT-dependent promoter. AT9283 inhibited STAT3-dependent luciferase activity with an EC50 of approximately 0.125 μM. Consistent with AT9283 induced cytotoxicity, genetic depletion of STAT3, AURKA or AURKB showed growth inhibition of MM cells, suggesting that AT9283-induced inhibition of these molecules is in part the underlying mechanism of MM cell growth inhibition. In vivo data using a xenograft mouse model of human MM show that mice treated with AT9283 demonstrated slower tumor growth compared to the control group (p=0.018) and prolongation in median overall survival (32 days in treated group versus 18 days in control group; p < 0.0001) without adverse effects. We next evaluated the activity of AT9283 in combination with established MM drugs and strong synergistic effect was found when AT9283 was combined with lenalidomide (Selleck Chemicals LLC, TX, USA) (Combination Index < 0.9). We hypothesized that the synergistic effect of this combination is due to the fact that the two drugs target different pathways and different phases of the cell cycle, thus augmenting their individual anti-myeloma activity. We examined MM cell cytotoxicity of the combination by using AT9283 and lenalidomide at concentrations lower than their maximal cytotoxic concentrations. Increasing doses of AT9283 (0 - 0.125 μM) were added to lenalidomide (0-2μM) and a significant decrease in viability (as measured by MTT and cell growth as determined by 3H-TdR at 48 h) was observed with combined therapy compared to either agent alone. A significant increase (55.7%) in early and late apoptosis occurred after 72 hours of exposure of cells to combined therapy with associated caspase-8 and PARP cleavage. Combination treatment resulted in downregulation of pSTAT3 and pERK following 4 hours of treatment. Considering the role that the BM microenvironment plays in growth and survival of MM cells, we examined whether the combination of low dose AT9283 plus lenalidomide induced MM cell death in the context of the BM microenvironment. MM.1S cells were cultured with or without BMSCs in the presence or absence of AT9283, lenalidomide or in combination regimen. Combined therapy inhibited 3H-TdR uptake of MM.1S cells cultured in the presence of BMSCs. Interestingly, we observed that AT9283 plus lenalidomide downregulated the expression of the p-STAT3 and p-ERK when MM.1S cells were cultured with BMSCs, highlighting the role of this drug combination in overcoming the protective effect of BMSCs. These results provide the rationale for the clinical evaluation of AT9283 in combination with lenalidomide in MM patients.

Abstract Number: 3930
**Presentation Title:** AT9283, a Novel Pan-Aurora/JAK-2 Kinase Inhibitor Suppresses Tumor Growth In Aggressive B-Cell Non-Hodgkin’s Lymphoma

**Presentation Time:** Monday, December 6, 2010: 6:00 PM-8:00 PM
**Poster Session:** Lymphoma – Pre-Clinical – Chemotherapy and Biologic Agents – Poster II
**Poster Venue:** Hall A3/A4 (Orange County Convention Center)
**Poster Board Number:** III-709

**Author Block:**

Daruka Mahadevan, MD, PhD\(^1\), Xiaobing Liu, BS\(^1\), Daniel Oscar Persky, MD\(^3\), Thomas P. Miller, MD\(^1\), Matthew S Squires, PhD\(^4\) and Wenqing Qi, PhD\(^2\)

\(^1\)Arizona Cancer Center, Tucson, AZ
\(^2\)Arizona Cancer Center, University of Arizona, Tucson, AZ
\(^3\)Clinical Medicine, Arizona Cancer Center, Tucson, AZ
\(^4\)Astex Therapeutics Ltd., Cambridge, United Kingdom

**Abstract Body:**

Aurora kinases (A and B) are oncogenic serine/threonine kinases that play a central role in regulating the mitotic phase of the eukaryotic cell cycle. Auroras are over-expressed in numerous tumors including aggressive B-cell non-Hodgkin’s lymphomas (B-NHL) and are considered validated oncology targets. AT9283 a pan-Aurora/JAK-2 inhibitor has undergone early phase trials in acute and chronic myeloid leukemia with promising anti-tumor activity. Hence, we hypothesized that 1) targeting mitosis with AT9283 would be effective in promoting apoptosis in aggressive B-NHL cell lines, and 2) addition of a microtubule targeting agent activating the spindle assembly checkpoint would lead to enhanced anti-tumor activity in a mouse xenograft model of mantle cell lymphoma (MCL).

AT9283 inhibited Aurora A and B activity as demonstrated by reducing phosphorylation of Aurora A and histone H3 as well as inhibition of cell proliferation (IC\(_{50}\) of 0.02-1.6 µM) in a variety of aggressive B-NHL cell lines (diffuse large B-cell lymphoma, MCL and transformed follicular lymphoma). B-NHL cells treated with AT9283 exhibited endoreduplication confirming the mechanism of action of an Aurora kinase inhibitor. Treatment of B-NHL cells with AT9283 induced apoptosis demonstrated by flow cytometry and PARP-cleavage in a dose and time dependent manner. Aurora A over-expression has been demonstrated to override the spindle assembly checkpoint (SAC) and result in resistance to microtubule targeting agent (e.g. taxols and vinca alkaloids) induced apoptosis. At very low doses (5-50 nM) apoptosis was almost doubled in the combination (30%) compared to AT9283 or docetaxel alone (15%) compared to controls. A mouse xenograft MCL model (Granta-519) demonstrated that AT9283 (10 and 15 mg/kg) or docetaxel (10 mg/kg) alone had modest anti-tumor activity (10-20% tumor growth inhibition). However, AT9283 plus docetaxel demonstrated a statistically significant tumor growth inhibition of >60% over control. The body weights of all mice in all cohorts did not change significantly (within 10%) during the study and mice appeared to tolerate treatment(s) well. Together, our results suggest that AT9283 plus docetaxel may represent a novel therapeutic strategy in aggressive B-cell NHL and warrant early phase clinical trial evaluation [Funded by the Lymphoma SPORE, P50 CA130805501A1].

**Abstract Number:** 1818

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**Presentation Title:** Adaptation and Validation of the Plasma Inhibitory Activity (PIA) Assay to Detect Inhibition of Aurora, ABL and FLT3 Kinases by AT9283 In Children and Adolescents with Leukaemia

**Presentation Time:** Saturday, December 4, 2010, 5:30 PM-7:30 PM
**Poster Session:** Molecular Pharmacology, Drug Resistance: Poster I
**Poster Venue:** Hall A3/A4 (Orange County Convention Center)
**Poster Board Number:** I-798

**Author Block:**

Jennifer E Podesta\(^1\), Melanie J Griffin\(^2\), Richard Sugar\(^3\), Matthew S Squires\(^4\), Alan Boddy\(^2\), Spiros Linardopoulos\(^5\), Andrew DJ Pearson\(^6\) and Andrew S Moore\(^6\)

\(^1\)Division of Surgery and Cancer, Imperial College, London, United Kingdom
\(^2\)Northern Institute for Cancer Research, Newcastle University, Newcastle upon Tyne, United Kingdom
\(^3\)Drug Development Office, Cancer Research UK, London, United Kingdom

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Abstract Body:

Non-invasive assessment of biomarker modulation is critically important when conducting early phase trials of targeted therapeutics, particularly in children. Measuring target modulation using primary leukaemic blasts is challenging, since obtaining bone marrow is invasive and invariably requires a general anaesthetic in children. Furthermore, in many cases only a limited number of circulating blasts can be detected in peripheral blood and these may be rapidly cleared upon commencement of effective therapy. For these two reasons, pharmacodynamic (PD) assays using surrogate biological material can be invaluable. The plasma inhibitory activity (PIA) assay was initially developed for FLT3 inhibitors and is currently used in clinical trials to assess ex vivo inhibition of FLT3 kinase and guide optimal dosing (Levis M et al, Blood 2006). Briefly, the assay involves incubating reference leukaemic cell lines in plasma from patients treated with FLT3 inhibitors then assessing the degree of FLT3 inhibition by western blotting. Inhibition of FLT3 in leukemia cell lines has been shown to correlate with inhibition in primary leukaemic blasts. Aurora kinase inhibitors are emerging as promising new agents with activity in leukaemia (Moore AS et al, Leukemia 2010). AT9283 is a novel Aurora kinase inhibitor with activity against the secondary kinase targets FLT3 and ABL (Howard S et al, J Med Chem 2009). We therefore hypothesised that the PIA assay would be applicable as an ex vivo PD assay to simultaneously detect inhibition of these target kinases in leukaemic cell lines: Aurora with FLT3 and Aurora with ABL. Furthermore, we hypothesised that the assay could be adapted to paediatric patients, where limited volumes of blood are available for PD studies. Here we report the validation of the PIA assay for use with AT9283 using leukaemic cell lines incubated in human plasma spiked with clinically relevant concentrations of AT9283 known to inhibit Aurora kinase in primary leukaemic blasts. The PIA assay proved to be a robust means of semi-quantitatively detecting concentration-dependent inhibition of Aurora kinase in the FLT3-ITD positive AML cell line MOLM-13 and the CML cell line K-562. The PIA assay was also effective at qualitatively detecting the inhibition of FLT3 signaling in MOLM-13 cells and ABL signaling in K-562 cells. Of importance to paediatric patients, the assay was successfully performed using 0.5 mL of plasma, half the volume described in the original PIA assay for FLT3 inhibitors. It will be used to assess target kinase modulation in a phase I trial of AT9283 in children and adolescents with relapsed and refractory acute leukaemia (EudraCT No. 2009-016952-36). In conclusion, the PIA assay is applicable not only to FLT3 inhibitors, but also Aurora kinase inhibitors and potentially, other multi-kinase inhibitors. By simultaneously detecting multiple kinase inhibition, the PIA assay may help delineate important mechanisms of action for novel anti-leukaemic drugs.