Encouraging data from three Astex collaborative programmes to be presented at the 101st American Association for Cancer Research (AACR) Annual Meeting 2010

Cambridge, UK, 13th April 2010

- **First disclosure of the chemical structure of a selective fibroblast growth factor receptor (FGFr) inhibitor lead series from Astex's collaboration with Janssen Pharmaceutica.**
- **Data from Astex's collaboration with the Dana Farber Cancer Institute and Massachusetts General Hospital demonstrating the unique JAK2 inhibitory activity of Astex's clinical stage kinase inhibitor, AT9283, supports its potential in the treatment of both myelofibrosis and multiple myeloma.**
- **Discovery of novel orally active, dual inhibitors of AKT and p70S6 kinase from Astex's collaboration with The Institute of Cancer Research.**

Astex Therapeutics, the UK-based biotechnology company developing targeted therapies for oncology, today announced presentations of promising new data from three of its collaborative programmes. The presentations will be made during the AACR 101st Annual Meeting, April 17-21, 2010, at the Walter E. Washington Convention Center in Washington DC, USA.

FGFr kinase inhibitors

Astex will chair the mini-symposium on "New Preclinical and Clinical Candidates" which will include the first disclosure of new anticancer agents from a number of research groups. Astex scientists will present new biological and pharmacological data on compounds from its FGFr inhibitor programme which was partnered with Janssen Pharmaceutica in June 2008 in a deal valued at more than $500M. Astex has used its fragment-based drug discovery approach to identify several lead series and an orally bioavailable, potent lead compound that is selectively active against FGFr. Astex's presentation at AACR represents the first disclosure of the chemical structure of a lead series and illustrates how a fragment-based drug discovery approach can be efficiently used to discover potent compounds with oral bioavailability. Fibroblast growth factor (FGF) and FGF receptor (FGFr) signalling are key targets in the molecular pathology of cancer.

AT9283 - a combinatorial oncogenic kinase inhibitor

Collaborative research being carried out by leading oncologists at the Dana Farber Cancer Institute and the Massachusetts General Hospital Cancer Center in Boston, has shown that Astex's combinatorial oncogenic kinase inhibitor, AT9283, has inhibitory activity against the key cancer target JAK2, that drives inhibition of growth of both myelofibrosis and multiple myeloma cell lines via the JAK/STAT pathway. These data clearly differentiate AT9283 from other compounds in the class. The significant activity of AT9283 in multiple myeloma cell lines provides the rationale for its clinical evaluation in this indication and Astex is currently planning for a Phase II study in patients with relapsed/refractory multiple myeloma in collaboration with the National Cancer Institute of Canada to commence in 2010.

AKT and p70S6 kinase inhibitors

In addition, new data will be presented describing the discovery of potent, orally active, dual inhibitors of AKT and p70S6 kinase - two key enzymes involved in tumour cell survival. The novel strategy of inhibiting both enzymes may have therapeutic value and the new data supports further evaluation of these compounds in patients with cancer. The compounds, AT7867 and CCT128930 are novel, ATP-competitive, AKT inhibitors developed at The Institute of Cancer Research, UK, in collaboration with Astex. AT13148, which is derived from the same lead series as AT7867, is currently completing preclinical development under a partnership between Astex, Cancer Research UK and Cancer Research Technology Limited that was announced in September 2008.

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About AT9283

AT9283 is a small molecule inhibitor of Aurora kinases A and B, with potent activity also against c-ABL and JAK2. Aurora kinases have been demonstrated to be over-expressed in several high risk cancers. Inhibitors of Aurora kinases, such as AT9283, represent attractive novel anti-cancer agents for the treatment of a broad range of solid tumours and haematological malignancies as evidenced by anticancer activity in tumour models and emerging early clinical data in adults. AT9283 has completed a Phase I study in patients with solid tumours and has been found to be well tolerated in a Phase I/IIa study in adult patients with haematological malignancies with early signals of efficacy in approximately one third of adult patients with relapsed/refractory acute myeloid leukemia. Further Phase I studies exploring alternative dosing regimens for the compound in solid tumour patients and in paediatric patients with solid tumours or with haematological malignancies are ongoing in collaboration with the National Cancer Institute of Canada and with Cancer Research UK, respectively.

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 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, GlaxoSmithKline, Novartis and Johnson & Johnson.

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

Editors Notes: AACR presentations

Abstract Number: 2530

Presentation Title: Antimyeloma activity of a small molecule multi-targeted kinase inhibitor, AT9283, via potent aurora kinase and STAT3 inhibition

Presentation Time: Monday, Apr 19, 2010, 2:00 PM - 5:00 PM
Location: Exhibit Hall A-C, Poster Section 22
Poster Section: 22
Poster Board Number: 17

Author Block: Loredana Santo[1], Teru Hideshima[1], Diana Cirstea[1], Erik A. Nelson[1], Madhavi L. Bandi[1], Gullu Gorgun[1], Sonia Vallet[2], Samantha Pozzi[1], Kishan Patel[2], Hiroshi Ikeda[1], Giulia Perrone[1], Yiguo Hu[1], Dharminder Chauhan[1], Matthew Squires[3], Nikhil C. Munshi[1], Kenneth C. Anderson[1], Noopur Raje[2]. [1]Dana Farber Cancer Institute, Boston, MA; [2]Massachusetts General Hospital Cancer Center, Boston, MA; [3]Astex Therapeutics Ltd, Cambridge, United Kingdom

Abstract Body:

Aurora Kinases are a family of mitotic regulators. Aurora Kinase A (AURKA) plays a crucial role in centrosome separation and spindle assembly and is required for mitosis and bipolar mitotic spindle formation. Aurora Kinase B (AURKB), a member of the chromosomal passenger complex, is required for chromosome segregation, spindle assembly checkpoint and cytokinesis. Both AURKA and AURKB are significantly overexpressed in MM cells, which has prompted the investigation of aurora kinase inhibitors as a therapeutic strategy in MM. Here, we investigated the preclinical activity of a small molecule multitargeted inhibitor, AT9283, with potent in vitro kinase activity against AURKA and AURKB kinases (3 nM), JAK2 and 3 (at 1.2 and 1.1 nM) and Abl T315I (at 4 nM). Growth inhibitory effects of AT9283 on MM cell lines and patient derived cells was observed with IC50 values of 0.25µM -0.5 µM at 48 hours using a [3H]thymidine incorporation assay. Cell cycle analysis following AT9283 treatment resulted in increased G2/M phase and polyploidy consistent with failed cytokinesis (associated with AURKB inhibition) confirmed by immunofluorescence assay. This was followed by induction of apoptosis assessed by Annexin V+PI+ staining peaking at 48 - 72 hours with associated -8-9 cleavage. Decreased levels of phosphorylated histone H3 at serine-10, a direct downstream substrate of AURKB, confirmed the role of AURKB inhibition by AT9283. Importantly, besides aurora kinase inhibition, we observed that AT9283 also inhibited STAT3 tyrosine phosphorylation in MM cells within 30 minutes of treatment. The effect of AT9283 on STAT3 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 ìM. Since MM cell lines with constitutive STAT3 tyrosine phosphorylation were more sensitive to AT9283, we investigated whether AT9283-induced effects on the JAK/STAT pathway correlated with Aurora inhibition. Genetic depletion by RNA interference showed that STAT3 knockdown in U266 cells did not affect the expression levels of AURKA and AURKB. In contrast, in cells with knocked-down AURK A and B, we observed a downregulation in the expression level of STAT3, due to either an off-target effect or the possibility that STAT3 is downstream of Aurora Kinases. Ongoing studies are aimed at

understanding whether AT9283-induced effects on the JAK/STAT pathway enhance the efficacy of aurora kinase inhibition in the context of MM. Finally, 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 without adverse effects. In conclusion, these results show significant anti-MM activity of AT9283, and provide the rationale for its clinical evaluation in MM.

Abstract Number: 3626

Presentation Title: Development of inhibitors of the fibroblast growth factor receptor (FGFR) kinase using a fragment based approach

Presentation Time: Tuesday, Apr 20, 2010, 9:00 AM -12:00 PM
Location: Exhibit Hall A-C, Poster Section 26
Poster Section: 26
Poster Board Number: 20

Author Block: Matthew S. Squires[1], Timothy Perera[2], Gordon Saxty[1], Chris Murray[1], Peter King[2], George Ward[1], Ruth Feltell[1], Sharna Rich[1], Patrik Angibaud[2], Edward J. Lewis[1], Ron Gilissen[2], Isobel Harada[1], Lynsey Fazal[1], Julie A. Irving[3], Mike A. Batey[3], Yan Zhao[3], David R. Newell[3], Neil T. Thompson[1]. [1]Astex Therapeutics, Cambridge, United Kingdom; [2]Ortho Biotech Oncology, Beerse, Belgium; [3]Northern Institute of Cancer Research, Newcastle, United Kingdom

Abstract Body:

Recent data in a number of tumour types has implicated Fibroblast Growth Factor (FGF) and Fibroblast Growth Factor receptor (FGFR) signalling as being key to the molecular pathology of cancer. FGFR is a receptor tyrosine kinase which activates the extracellular signal-regulated kinase / mitogen-activated protein kinase and the protein kinase B / Akt pathways which promote cell growth and survival. Amplification, over-expression or activating mutations of fibroblast growth factor receptors have been associated with bladder tumours, multiple myeloma, hormone-refractory prostate cancer and breast cancer.

Multiple lead series of FGFR inhibitors were developed using Astex's fragment based medicinal chemistry approach, Pyramid™, linked to high throughput-X-ray Crystallography. We describe here the characterisation of some examples of these lead molecules. In particular we detail the pharmacological profile of a compound from one of these lead series that demonstrated activity against FGFR 1-4 with an IC50 <100nM in an isolated kinase assay. This compound inhibited FGFR1-4 kinase activity in BaF3 cell lines engineered to express the relevant kinase fusion proteins and proliferation and survival of a panel of FGFR-dependent human tumour cell lines derived from several different tissues. The cytotoxic activity was >10 fold lower in cell lines lacking FGFR expression. We demonstrate inhibition of FGFR 2 and 3 phosphorylation in gastric and multiple myeloma cell lines respectively with associated inhibition of downstream signalling pathways.

This lead molecule has an excellent pharmacokinetic profile and high oral bioavailibility in mice and rats. In xenograft models in mice where aberrant FGF signalling underlies tumour pathology, tumour growth inhibition is observed at doses of 100mg/kg /day orally for 21 days. This xenograft efficacy was observed in several models, with significantly lower activity in models where aberrant FGF signalling is not involved in tumour pathology. This suggests that the mechanism of action is consistent with FGFR inhibition. The pharmacological profile in these models is also distinct from other broader spectrum receptor tyrosine kinase inhibitors.

The pre-clinical data shown here suggests that such compounds warrant further investigation pre-clinically and may benefit patients whose disease is driven by FGFR activity.

Abstract Number: 4481

Presentation Title: First report of preclinical pharmacology of two novel potent AKT inhibitors and development of pharmacodynamic (PD) biomarkers in tumor and surrogate tissue

Presentation Time: Tuesday, Apr 20, 2010, 2:00 PM - 5:00 PM
Location: Exhibit Hall A-C, Poster Section 24
Poster Section: 24
Poster Board Number: 16

Author Block: Timothy A. Yap[1], Mike I. Walton[1], Lisa Hunter[1], Kyla Grimshaw[2], Melanie Valenti[1], Paul Eve[1], Simon P. Heaton[1],

Lisa Pickard[1], John J. Caldwell[1], Neil Thompson[2], Johann S. de Bono[1], Stan B. Kaye[1], Sue A. Eccles[1], Paul Workman[1], Ian Collins[1], Michelle D. Garrett[1]. [1]The Institute of Cancer Research, Sutton, United Kingdom; [2]Astex Therapeutics, Cambridge, United Kingdom

Abstract Body:

Deregulated AKT signalling is implicated in cancer. The preclinical characterization of AKT inhibitors and development of PD biomarkers are vital prior to clinical trials. Invasive techniques for clinical PD analyses pose ethical and logistical issues, hence hair follicles may represent a non-invasive option. The pyrazole AT7867 and pyrrolopyrimidine CCT128930 are novel ATP-competitive AKT inhibitors from different chemical series developed at The Institute of Cancer Research in collaboration with Astex Therapeutics (Cambridge, UK).

AT7867 and CCT128930 have IC50 values against AKT2 of 17nM and 6nM respectively. Growth inhibitory IC50 (GI50) values were 2.4ìM and 6.3ìM in PTEN-null U87MG glioblastoma cells as measured by SRB assay. Cellular studies of both compounds in U87MG cells were carried out by Meso Scale Discovery (MSD) ELISA, western blot (WB) and immunofluorescence. These showed an initial induction of phosphorylated (p) Ser473 AKT as expected with ATP-competitive AKT inhibitors, and inhibition of downstream AKT targets, including pSer9 GSK-3â, pThr246 PRAS40, pSer127 YAP and pSer235/236 S6RP, indicating AKT pathway blockade. Interestingly, CCT128930 inhibited phosphorylation of AKT targets GSK-3â, S6RP and PRAS40 at lower concentrations (0.5-1µM), compared to AT7867 (5-10µM) in U87MG cells. Phosphorylation was also inhibited at earlier time points with equipotent doses (3XGI50) of CCT128930 versus AT7867. CCT128930 also caused a predominant G0/G1 phase arrest, while AT7867 resulted in a predominant G2 arrest in U87MG cells at equipotent doses (3XGI50), using BrdU and PI staining and flow cytometry. We report for the first time in vivo efficacy with intraperitoneally (ip) or orally administered AT7867 in PTEN-null MES-SA uterine sarcoma and U87MG mouse xenograft models; and ip administered CCT128930 in HER2-overexpressing BT474 breast cancer and U87MG xenografts, which correlated with in vivo pharmacokinetics (PK) and PD biomarker modulation of pSer9 GSK-3â and pSer235/236 S6RP using WB and MSD.

Hair follicles were developed as a robust surrogate PD biomarker, with pThr246 PRAS40 as a PD readout. This assay was validated with healthy volunteer hair follicles, which were treated ex vivo with CCT128930. A significant decrease in pThr246 PRAS40 ($p<0.001$) relative to total PRAS40 was observed with immunofluorescence, which was quantified using INCell Translator software. This assay is currently being utilized as a PD readout in the MK2206 Phase I AKT inhibitor trial, and will be used in future clinical studies of ATP-competitive AKT inhibitors.

In conclusion, by employing an integrated PK and PD biomarker-driven drug discovery strategy, we have developed 2 novel and potent AKT inhibitors with antitumor activity, and have validated a robust surrogate biomarker assay for AKT inhibition for use in clinical trials.

Minisymposium: MS.CH01.01. New Preclinical and Clinical Candidates. Wed, April 21, 09:30-12:00pm

Abstract Number: 5778

Presentation Title: Fragment-based drug discovery of selective inhibitors of fibroblast growth factor receptor (FGFr)

Presentation Time: Wednesday, Apr 21, 2010, 10:10 AM -10:25 AM
Location: Room 102, Washington Convention Center

Author Block: Gordon Saxty[1], M. S. Squires[1], C. W. Murray[1], V. Berdini[1], G. A. Ward[1], D. Miller[1], S. J. Rich[1], A. Cleasby[1], S. M. Saalau-Bethell1, J. Coyle1, A. Madin[1], M. G. Carr[1], M. A. O'Brien[1], C. Griffiths Jones[1], E. Vickerstaff[1], R. K. Nijjar[1], B. Graham[1], A. Pike[1], E. J. Lewis[1], T. Perera[2], P. Angibaud[2], H. Newell[3]. [1]Astex Therapeutics, Cambridge, United Kingdom; [2]Ortho Biotech Oncology R&D, Beerse, Belgium; [3]Northern Institute for Cancer Research, Newcastle, United Kingdom

Abstract Body:

Recent data in a number of tumour types has implicated Fibroblast Growth Factor (FGF) and Fibroblast Growth Factor receptor (FGFr) signalling as being key to the molecular pathology of cancer. This poster will describe fragment based drug discovery using biophysical screening to identify initial fragments. Subsequently, in the fragments-to-leads stage a detailed structural understanding of the binding interactions between the fragment and its target protein utilised X-ray crystallography and NMR. Starting with different fragments allows several lead series to be identified, often by synthesizing only small numbers of compounds.

A fragment screening campaign was conducted against the FGFr-1 to detect very low molecular weight compounds that bound

to the hinge region of the kinase. The screening produced several fragment molecules (Molecular Weight <250 Da) which were in the micromolar range and confirmed binding mode in X-ray crystallography. One X-ray hit series that was 120 uM verse FGFr-3 will be described. Several iterations of structure-guided medicinal chemistry led to the identification of a lead compound with 3 nM affinity for FGFr-3, good cell activity and 30-fold selectivity verse VEGFr-2 with good oral activity. The lead was optimised to afford a compound that showed good PK/PD and efficacy.

This poster represents first disclosure of the structure of the lead series and illustrates how a fragment-based drug discovery approach can be efficiently used to discover compounds advanced nanomolar compounds with oral bioavailability.