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Pharmacokinetic-pharmacodynamic (PK-PD) modeling of tumor growth inhibition in mice: the activity of brostallicin is enhanced in cells with high glutathione-S-transferase

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Brostallicin (PNU-166196) is a α-bromoacrylic distamycin-like derivative DNA minor groove binder, currently in Phase II clinical evaluation. The compound showed a broad spectrum antitumor activity in preclinical models. In particular, it was found that the antitumor activity of brostallicin was higher in the glutathione-S transferase-π (GST-π) overexpressing tumors without increased toxicity. In this communication we used a PK-PD model for evaluating the quantitative relationship between brostallicin activity and GST-π expression.

In this PK-PD modeling approach the growth of tumors in non-treated animals is described by an exponential phase followed by a linear growth phase. The tumor growth in treated animals is considered decreased by a factor proportional to both plasma drug concentrations and weight of proliferating tumor cells. A transit compartmental system is used to model the delayed process of cell death. The parameters of the pharmacodynamic model are related to the drug potency, the kinetics of the tumor cell death, and the growth characteristics of the tumor (Simeoni et al, Cancer Res. 64:1094; 2004).

Nude mice were inoculated with GST-π-transfected versus empty vector-transfected A2780 human ovarian carcinoma clones. Clones were characterized for their expression of GST-π. When the tumor was palpable, the animals were randomized to receive either placebo or brostallicin. Tumor mass was timely evaluated using standard caliper measurements. Plasma PK of brostallicin were obtained from an ancillary experiment. Simultaneous fitting of the average tumor mass in control and treated animals was performed using non-linear regression (Winnonlin 3.1, Pharsight).

Model fittings were good and provided reliable estimates of K2, the parameter describing the potency of brostallicin.

A2780 clone	GST-π expression (nmol/min/mg protein)	Potency K2 ($\mu M^{-1}day^{-1}$)	CV (%)
16	13.6	9.00	24.1
7	25.0	18.74	43.5
8	30.7	16.77	14.6

As can be noticed a doubling in the GST-π expression was reflected in a doubling of K2. This PK-PD modeling approach provides parameters that can be more easily correlated to other experimental covariables than the typical metrics (e.g., %tumor growth inhibition) used to describe the activity of anticancer drugs in *in vivo* experiments.

114 POSTER

Cell lysate arrays – High performance protein profiling and signaling pathway mapping

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Mapping of signaling pathways while studying biological systems is one of the key areas of interest in genomic and proteomic research. In genomic research, the parallel analysis of differential expression levels has driven DNA microarrays to become a routine method to find and validate relevant markers for specific biological effects. Novel technologies for hypothesis-driven studies in proteomics research, such as protein microarrays, now allow to quantify subtle changes of protein expression or protein activation (e.g. phosphorylation, methylation, acetylation) in cellular systems, thus expanding the range of conventional analytical techniques (e.g. Western blotting) which are facing the limitations of throughput and economy-of-scale. In addition, the high sensitivity of microarrays will become of utmost importance when the amounts of sample material are limiting (e.g. biopsies, micro-dissection).

Zeptosens has developed ZeptoMARK® CeLyA assays using cell lysate arrays. Cell lysate arrays have been introduced for rapid routine profiling of proteins, featuring high sensitivity (capable to detect 600 protein molecules per microspot), high throughput (up to 264 sample spots/array, up to 360 arrays/run) and economic use of reagents. The reverse array format involves the spotting of crude cell extracts onto the chip surface and the use of high specificity antibodies to detect the target proteins or protein modifications of interest. Cell lysate arrays are preferentially applied when larger numbers of samples than numbers of protein targets are to be analyzed.

Examples of ZeptoMARK® CeLyA applications in drug profiling and pathway mapping of different cell lines, as well as examples of phospho-proteome profiling using clinical samples (e.g. cancer tissues), will be presented. The achieved high array-to-array and chip-to-chip signal reproducibility allowed the quantitative detection of 10–20% changes in protein expression and activation levels. Only within days, results could be provided, which were in good agreement with researcher's expectations on biological events and showed a good correlation with conventional Western blotting data. Compared with Western blotting, a benefit factor of more than 100 in saving time, labor and reagent volumes could be demonstrated.

Drug design and synthesis

115 POSTER

A radioiodinated meta-iodobenzylguanidine-octreotate conjugate

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Successful treatment of neuroblastoma (NB) with meta-[^{131}I]iodobenzyl-guanidine (MIBG) is compromised by the relatively short residence time of the radioactivity in tumor. NB and other tumors express somatostatin receptors (SSTR), offering the possibility of developing a bispecific therapeutic. The residence time of [^{131}I]MIBG in NB might be improved by conjugating it with an SSTR-avid peptide, octreotate, that will direct the radioactivity to the lysosomes. MIBG-octreotate (**5**) and precursors with bromine (**4**) and tin (**6**) moieties were synthesized as shown in Figure 1.

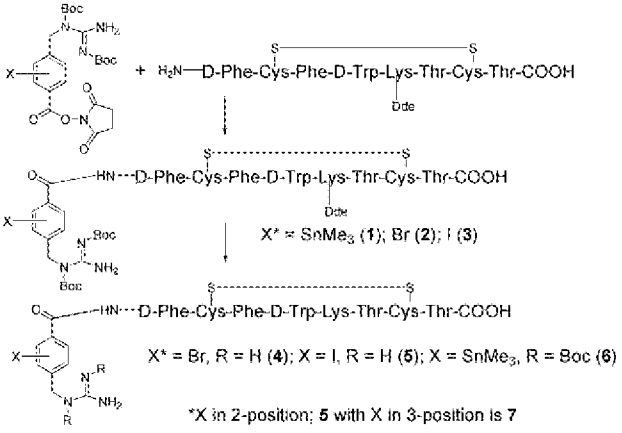

Figure 1. Scheme for synthesis of N$^{\alpha}$-(4-guanidinomethyl-2-[^{131}I]benzoyl-D-Phe^1-Octreotate (MIBG-Octreotate; **5**) and N$^{\alpha}$-(4guanidinomethyl-3-[^{131}I]-benzoyl-D-Phe^1-Octreotate (OIBG-Octreotate;**7**).

Attempts to synthesize [^{131}I]**5** by radioiodinating **6** were futile. [^{131}I]**5** was synthesized from **4** albeit in 5–10% radiochemical yields. The uptake kinetics of [^{131}I]**5** and [^{125}I]MIBG were compared in $SSTR_2$-expressing SH-SY5Y and SK-N-SHsst2 (Regulatory Peptides, 2000; 88: 61) NB cell lines. Studies using SK-N-SHsst2 cells also were performed to 1) to compare the uptake of [^{131}I]**5** with that of [^{125}I]GluTOCA (Clin. Cancer Res., 2003; 9:1868) and OIBG-Octreotate (**7**), and 2) to compare its washout with that of [^{125}I]MIBG. About 50% of input dose of [^{125}I]MIBG was taken up by both cell lines at 4h. The uptake of [^{131}I]**5**, compared to that of [^{125}I]MIBG, was about 2- to 3-fold lower in SK-N-SHsst2 cells; it was even lower in the SH-SY-5Y line. Desipramine (DMI), which inhibits the norepinephrine transporter (NET)-mediated Uptake-1, abrogated the uptake of [^{125}I]MIBG but not that of [^{131}I]**5** suggesting that the uptake of **5** in these two cell lines must be predominantly related to the SSTR2 binding. In SK-N-SHsst2 cells, [^{131}I]**5** had a higher whole cell uptake than [^{125}I]GluTOCA with a greater than 10-fold difference at 4 h; 1 μM octreotide reduced its uptake to 15% of the control. After allowing the SK-N-SHsst2 cells to accumulate [^{131}I]**5** and [^{125}I]MIBG for 4 h, their ability to retain the radioactivity was determined. For [^{125}I]MIBG, 60% of radioactivity was washed out within 4 h; however, the remaining activity was retained up to 48 h. The radioactivity from [^{131}I]**5** was released at a slower rate with the cells having higher amounts of

initially bound ^{131}I activity up to 24 h compared to that for ^{125}I. In SK-N-SHsst2 cells, the specific internalized radioactivity of [^{131}I]**5** was 2- to 4-fold higher than that of [^{125}I]GluTOCA. It was 6- to 7-fold higher than that of [^{125}I]**7** suggesting that, contrary to observations above, NET may have some role in its uptake (OIBG has no significant affinity to NET). In summary, while [^{131}I]MIBG-Octreotate demonstrated higher internalized radioactivity in SK-N-SHsst2 cells *in vitro* compared to [^{125}I]Glu-TOCA and [^{125}I]OIBG-Octreotate, its uptake was lower than that of [^{125}I]MIBG. We plan to modify the linker between MIBG and octreotate in order to facilitate the interaction of this hybrid molecule with both tumor associated targets present on NB cells.

116 POSTER

Inhibition of p53-MDM2 pathway by novel boronic-chalcones

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The p53 tumor-suppressor pathway is inactivated in a majority of human cancers. Although the p53 gene is frequently deleted or mutated in many human malignancies, a substantial percentage of tumors also express intact p53 and overexpression of MDM2 is commonly observed. The oncoprotein MDM2 negatively regulates p53 function by binding to this protein to enhance proteolytic degradation, hence destroying the cell cycle checkpoint and allowing the progression of damaged cells. This P53/MDM2 interaction has been implicated as a possible mechanism for cancer development in several tumors including human sarcomas. Thus disruption of p53-MDM2 interaction with synthetic compounds should stabilize p53 in the nucleus and offer a novel therapeutic potential for cancer therapy. A series of boronic-chalcones have been investigated as possible MDM2 antagonists. The goal of the current studies is to build upon the paradigm of the boronic-chalcone analogs to identify more effective and selective agents can be found. We have successfully designed and synthesized boronic-chalcone derivatives that inhibit growth of human breast and colorectal cancer cell lines with IC_{50} values from 1 to 5 μM. The cytotoxic effect of these compounds was measured by multiple analyses including MTT assay, annexin-V reactivity, and colony formation assay. Both apoptosis analysis and colony formation assay in p53 isogenic cells showed that the p53+/+ colon cancer cells are more sensitive to the active boronic-chalcones than the p53−/− colon cancer cells. We have shown by multidimensional NMR spectroscopy that boronic-chalcone derivatives are MDM2 inhibitors that bind to a subsite of the p53-binding cleft of human MDM2. Structure-activity relationship studies and molecular modeling studies of this new class of compounds are underway. Upon the identification of the most active compounds, these cytotoxic agents will be tested for their potency and selectivity for tumor cells. The lead compounds will then be tested in vivo models of human breast and colon cancer. These studies will serve to identify the best candidate that will subsequently test in clinical trials as treatment for breast and colon cancer.

117 POSTER

Identification of novel cyclin dependent kinase 1/2 inhibitors using fragment based high-throughput X-ray crystallography and structure based drug design

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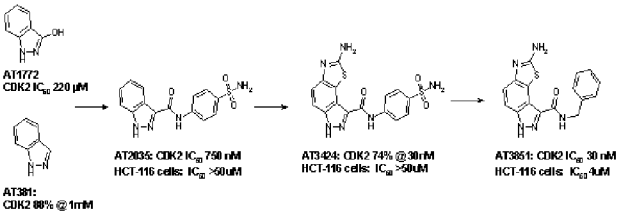
The poster describes the use of high-throughput X-ray crystallography and fragment-based drug discovery (Astex's Pyramid™ technology) to develop a number of lead series with potent cyclin-dependent kinase 1 and 2 (CDK-1, CDK-2) inhibitory activity and antiproliferative activity against cancer cell lines.

Astex has developed an integrated crystallography-based approach, which allows the detection of high efficiency binding molecular fragments and their subsequent optimization using structure-based drug design into potent novel drug candidates. Soaking apo-crystals of CDK-2 with cocktails of low molecular weight compounds identified a number of start points for chemistry programmes. Optimisation of one of these start points, using X-ray structures of synthesized molecules, allowed the rapid identification of compounds with potent CDK activity. Further improvements in the initial leads have identified compounds with both potent CDK and single figure nanomolar anti-proliferative activity.



These lead molecules were characterised in a range of cell-based assays, demonstrating their anti-proliferative effect resulted from a specific cell cycle arrest and tumour cell death by apoptosis. The mechanism of action of this inhibition was confirmed by monitoring the phosphorylation of downstream substrates.

Furthermore the compounds were shown to exhibit negligible toxicity towards non-proliferating fibroblast cells, and were equipotent in cells lacking p53 or expressing PgP.

The in vivo pharmacokinetic and xenograft activity of this series of compounds will be described in the accompanying poster.

In conclusion using Astex's Pyramid™ technology a number of potent CDK1/2 inhibitors have been identified with potent anti-tumour activity.

118 POSTER

Substituted 7-amino-4-anilino-6-alkoxy-3-quinolinecarbonitriles as Src kinase inhibitors

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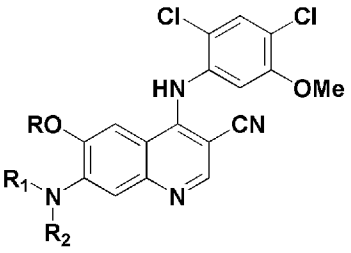
As a prototype for non-receptor tyrosine kinases and proto-oncogenes, Src plays an important role in the signal transduction pathways that regulate several cellular functions such as proliferation, differentiation, migration, and angiogenesis. Activation and over-expression of Src have been implicated in cancer, osteoporosis and stroke. Therefore, inhibition of Src kinase could prove effective in the treatment of these diseases. Earlier, a Wyeth team reported 7-alkoxy, 7-alkenyl, 7-alkynyl, and 7-phenyl-4-anilino-3-quinolinecarbonitriles as potent Src kinase inhibitors. In this paper, we report a series of substituted 7-amino-4-anilino-3-quinolinecarbonitriles. Some of them are low nanomolar inhibitors of Src kinase, and their SAR will be discussed. Methods for introducing substituents with various chain lengths on the 7-amino group will also be presented.



119 POSTER

A novel strategy to inhibit Stat3 for human cancer therapy

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Background: Stat3 has been suggested as a critical mediator of oncogenic signaling in the development and progression of human cancers and is active in prostate cancers (82%), breast cancers (69%), head and neck cancers (HNSCC) (>90%), nasopharygeal carcinoma (71%) as well as in many other cancers. Several Stat3 regulated genes, such as Bcl-x and Mcl-1, play important roles in cancer progression. Despite a strong rationale for targeting Stat3 for the treatment of human cancers, current chemotherapeutic approaches have not yet incorporated this strategy. We propose a novel strategy to inhibit Stat3, which should be useful in the development of novel cancer therapeutic approaches.

Methods: To design a novel inhibitor of Stat3, we employed several procedures: (1) structure-based drug design and optimization based upon our newly established model of drug/Stat3 complex and a structure-activity relationship (SAR) between inhibitors and Stat3, (2) chemical synthesis,