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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.

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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

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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.

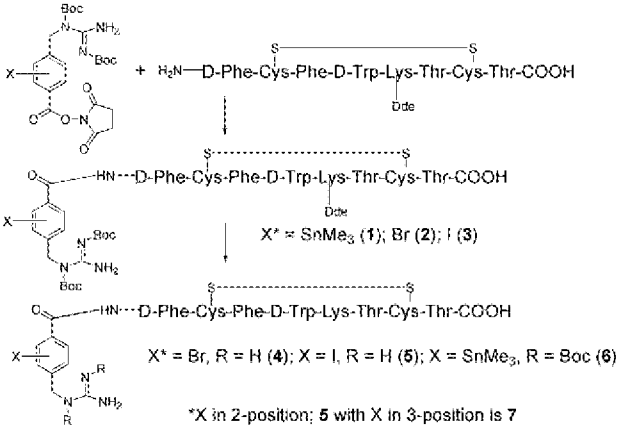

Figure 1. Scheme for synthesis of N$^{\alpha}$-(4-guanidinomethyl-2-[^{131}I]benzoyl-D-Phe^1-Octreotate (MIBG-Octreotate; **5**) and N$^{\alpha}$-(4guanidinomethyl-3-[^{131}I]-benzoyl-D-Phe^1-Octreotate (OIBG-Octreotate;**7**).

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