SP-083
Development of versatile dual-modality imaging probes based on a GRPR antagonist for preoperative imaging and image-guided surgery of prostate cancer

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Objective: The multifocal nature of prostate cancer often leads to partial removal of the tumor lesions during surgery [1]. Molecular imaging with dual-modality imaging probes could greatly improve prostate cancer management by providing more information about the localization of the primary tumor and the metastases, and by better defining surgical margins. Such probe bears a radionuclide to enable diagnosis via positron emission tomography (PET) and a fluorescent dye for image-guided surgery. Gastrin releasing peptide receptor (GRPR) is upregulated in the majority of the prostate cancer cells, and it has been identified as a promising target for imaging and treatment of prostate cancer. NeoB is a GRPR antagonist, which has been successfully labeled with gallium-68 and lutetium-177 for theranostic applications [2,3]. The main objective of this project was to develop a series of dual-modality imaging probes based on the molecular backbone of NeoB and bearing a DOTA chelator to enable labeling with a radiometal for preoperative PET imaging and a fluorescent dye for intraoperative surgical guidance.

Methods: Four NeoB analogs were synthesized by solid-phase peptide synthesis (SPPS). A DOTA chelator and a *trans*-cyclooctene (TCO) were selectively introduced at the *N*-terminus of the peptides. The final probes were obtained by performing a "click reaction" based on the inverse electron demand Diels-Alder reaction to couple a tetrazine-modified sulfo-cyanine 5 derivative (Tz-sCy5) to the TCO-peptides. The final products were purified by semi-preparative HPLC and characterized by MS. Indium-111 radiolabeling and stability studies were carried out, followed by biological assays to determine the binding affinity (IC_{50}) of the probes. Biodistribution and imaging studies were performed in GRPR-positive tumor bearing mice with the best two dual-labeled probes.

Results: The IC_{50} values of the four dual-labeled probes were 5 to 25 folds lower than the binding affinity of the parent peptide, which was expected due to the chemical modifications taking place. SPECT/CT imaging and an *ex vivo*biodistribution were performed at 2 and approximately 3 h post injection, respectively. Both probes showed high tumor uptake with 8.47±0.46 for DOTA-K(PEG_4-TCO-Tz-sCy5)-*p*ADA-BD (probe **12**) and 6.90±0.81%ID/g for DOTA-K(TCO-Tz-sCy5)-PEG_4-BD (probe **15**). Co-localization of the radioactivity and fluorescence was confirmed by *ex-vivo* fluorescent imaging.

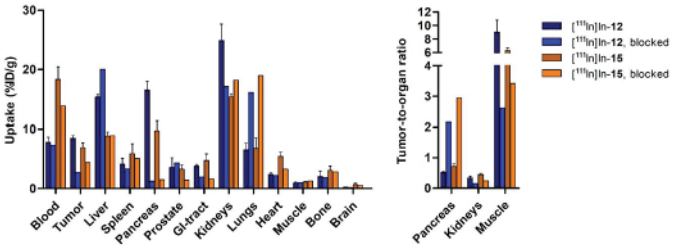

Fig. 1 (abstract SP-083).

Conclusion: Our dual-labeled probes demonstrated very promising biodistribution profile. They are promising drug candidates for preoperative diagnosis and intraoperative surgical guidance of GRPR-positive prostate cancer.

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Short Presentations: Nanomedicine

SP-084
Au@Pt core-shell nanoparticle bioconjugates for Auger electron radiotherapy – preliminary studies

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Objectives: Despite the broad development of medicine for cancer treatment, current therapeutic approaches are not efficient at dealing with aggressive and therapy-resistant neoplasms such as breast cancer or hepatocellular carcinoma. In these tumors, one of the most difficult steps of the therapy process is metastases treatment due to the spread of small size tumors. For this approach, targeted Auger therapy is one of the most promising options and platinum-based radiopharmaceuticals, due to their suitable characteristics, are promising candidates for realizing "chemo-Auger" therapy which is dedicated to small tumors and metastases.

Methods: This presentation summarizes the first part of research on the applications of core-shell (Au@Pt) nanoparticles for Auger therapy of HER2+ breast cancer and hepatocellular carcinoma. Two therapeutic approaches effectiveness was evaluated – targeted breast cancer therapy (bioconjugate Au@Pt-PEG-Trastuzumab) and nanobrachytherapy of hepatocellular carcinoma (Au@Pt-PEG-COOH). Synthesized conjugates were evaluated for *in vitro* studies such as binding affinity, internalization and cytotoxicity with conventional (2D) and spherical SKOV-3 and HepG2 cell cultures. Localization of the conjugates in the cell was visualized with confocal microscopy imaging. To verify the mechanism related to the solubility of PtNPs in the cytoplasm and responsible for platinum cytotoxicity in HepG2 cells, the platinum concentration in isolated cell nuclei was determined using ICP-MS. Using General Oxidative Stress Indicator, reactive oxygen species (ROS) concentration in treated cells was established.

Results: Studies carried out on the SKOV-3 cell line with the use of a synthesized bioconjugate revealed a high affinity to HER2+ cells, its over 90% internalization, placement in the perinuclear area and partial intranuclear location. During the cytotoxicity studies any effect was observed, what is probably related to the low concentration of platinum atoms in the Au@Pt-PEG-trastuzumab bioconjugate and not enough ROS concentration in the cells tested. As was found, Au@Pt-PEG-COOH was successfully internalized into HepG2 cells. Comparable cytotoxicity studies for SKOV-3 and HepG2 cell lines with mono- (10 µg Pt/mL) and multilayer Au@Pt (145 µg Pt/mL) were performed and no toxicity was observed for the SKOV-3 cell line in both concentrations. The results were quite different for liver cancer cells where multilayer Au@PtNPs induced toxicity with a gradual decrease of metabolic activity of cells after 24-72 hours. Unfortunately, significant Pt transport to the cell nucleus was not noted.

Conclusions: Synthesized conjugates were studied in terms of their application as radioactive analogues labeled with ^{193m,195m}Pt.

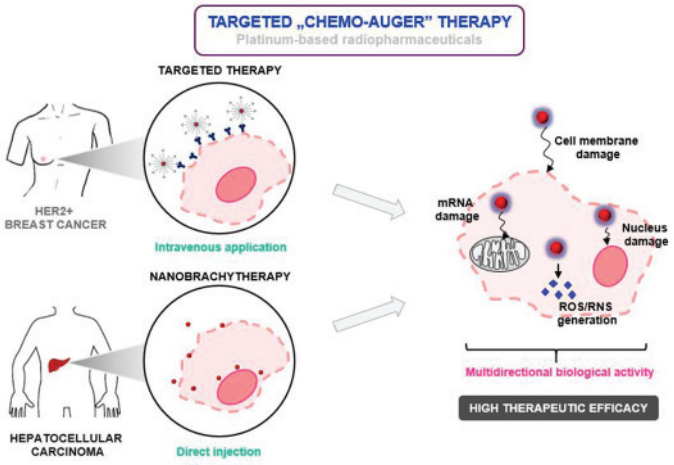

Fig. 1 (abstract SP-084).

When radioactive ^{193m,195m}Pt emitting Auger electrons are applied, it is reasonable to expect a cytotoxic effect for SKOV-3 cells due to the perinuclear location and probable partial intranuclear location. In the studies related to HCC therapy it was found, that after successful internalization of conjugate, the cytotoxic effect is associated with the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS). The obtained results are promising for further development of Au@^{193m,195m}Pt nanoparticles conjugates as a novel Auger electron-emitting radiation nanomedicine.

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SP-085
Development of discoidal phospholipid bilayer nanoparticles with styrene maleic acid copolymer for diagnosis and therapy of intractable cancers

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Objectives: Various types of lipoprotein-mimicking nanoparticles have been developed for delivering cancer therapeutic and diagnostic agents. Recently, we reported high density lipoprotein-like discoidal phospholipid bilayer nanoparticles (nanodiscs) with a biocompatible synthetic polymer, stylene maleic acid copolymer (SMA). SMA takes the place of apolipoproteins which can produce nanodiscs by mixing with phospholipids. Compared to liposomes with diameters of about 100 nm, SMA nanodiscs with diameters of about 10 nm showed a slow blood clearance and low accumulation in the spleen [1]. In this study, we focused on the reduced sizes of SMA nanodiscs, because reduced sizes of nanoparticles would be effective for the accumulation in intractable cancers such as pancreatic cancer characterized by low vascularization and reduced vascular permeability [2]. Then, the effects of types of phospholipids on the particle sizes and biodistribution of SMA nanodiscs were evaluated in normal mice, and tumor accumulation of the SMA nanodiscs was investigated in mice inoculated with tumors characreized by increased and reduced vascular permeability.

Methods: SMA was mixed with 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC), or 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC) to form SMA nanodiscs, followed by purification with gel filtration chromatography. Size distributions of SMA nanodiscs were determined using dynamic light scattering measurements. The SMA nanodiscs containing 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-diethylenetriamine- pentaacetic acid (1.0 mol%) were labeled with ^{111}In in citrate buffer. In biodistribution analyses, ^{111}In-labeled DMPC-, POPC-, and DSPC-SMA nanodiscs were intravenously administered to normal mice (10 nmol total lipids/mouse, 37 kBq/mouse). At 1, 6, and 24 hours after administration, the radioactivities in the tissues of interest were measured. In mice inoculated with tumors characreized by increased and reduced vascular permeability (colon26 and BxPC-3), biodistribution of DMPC-SMA nanodisc and DMPC liposome (control) was determined at 24 hours post-injection.

Results: The particle sizes and polydispersity index of DMPC-, POPC-, and DSPC-SMA nanodiscs were 6.9, 9.8, 25.0 nm, and 0.348, 0.549, 0.218, respectively. All SMA nanodiscs were successfully labeled with ^{111}In with high radiochemical yields (>80%) and purities (>95%). In normal mice, radioactibity in the blood after administration of DMPC-, and POPC-SMA nanodiscs were higher than that of DSPC-SMA nanodisc, and higher accumulation was obserbed in the liver and spleen of DSPC-SMA nanodisc-administered mice. These results may be due to an increased particle size of DSPC-SMA nanodisc. From the narrow dispersity and slow blood clearance, biodistribution of DMPC-SMA nanodisc was evaluated in tumor-bearing mice. Tumor to blood ratios of DMPC-SMA nanodisc at 24 hours post-injection were relatively high (colon26, 7.8; BxPC-3, 4.1). In addition, the BxPC-3 to colon26 ratio of DMPC-SMA nanodisc was higher than that of DMPC liposome (0.55±0.22 *vs* 0.41±0.20). These results suggested that the DMPC-SMA nanodisc showed improved accumulation in the intractable pancreatic cancer.

Conclusions: The DMPC-SMA nanodiscs would be effective nanocarriers for the disgnosis and therapy of intractable cancer.

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SP-086
Development of a radiolabeled self-assembled nanoparticle as an imaging probe for CD44-expressing tumors

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Objectives: CD44, a well-known cancer stem cell marker, drives tumor metastasis and progression and brings poor prognosis. CD44 is thus considered to be an attractive target for evaluation of tumor malignancy. Since CD44 is a receptor for hyaluronic acid (HA), HA can be a targeting moiety for CD44-expressing tumors. Previously, we have reported a biocompatible ternary anionic complex composed of indium-111 (^{111}In)-labeled polyamidoamine dendrimer (4th generation; G4), polyethyleneimine (PEI), and g-polyglutamic acid (g-PGA) prepared on the basis of electrostatic interaction, which succeeded in the visualization of macrophage-rich sentinel lymph nodes by single-photon emission computed tomography (SPECT) [1]. In this study, we newly prepared a ternary self-assembled complex using ^{111}In-DTPA-G4, PEI, and HA instead of g-PGA, and evaluated its effectiveness as an imaging probe for CD44-expressing tumors.

Methods: G4 was modified with a metal chelator, *p*-SCN-Bn-DTPA, thereafter ^{111}In-labeling was carried out by reacting DTPA-G4 with $^{111}InCl_3$. We constructed a ternary complex ^{111}In-DTPA-G4/PEI/HA without purification at a theoretical charge ratio; carboxyl groups of ^{111}In-DTPA-G4: amino groups of PEI: carboxyl groups of HA=1:8:32. Cellular uptake of ^{111}In-DTPA-G4/PEI/HA in T24 cells