magnetic resonance contrast agent Mn^{2+}, and labeled long half-life positron nuclide ^{64}Cu with PET and MRI imaging function. Cell uptake with the U87MG and A549 cell lines to evaluate the specificity of the (^{64}Cu, Mn)-FAP-PEG-MNPs nanoprobe. The pharmacokinetics, and PET or MRI imaging of the (^{64}Cu, Mn)-FAP-PEG-MNPs nanoprobe were researched in healthy Kunming (KM) and/or U87MG or A549 model mice.

Results: The radiochemical purity of the positron nuclide ^{64}Cu to FAPI-PEG-MNPs was over 90%, and the stability was good in vitro. Cell blocking experiment showed that the uptake ratio of (^{64}Cu, Mn)-FAP-PEG-MNPs nanoprobe in the U87MG cell blocking group and the blocking group was 3.34 ± 0.75 % and 0.42 ± 0.19 %, respectively at 2 h. In vitro inhibition of uptake of (^{64}Cu, Mn)-FAP-PEG-MNPs in U87MG cells preliminarily proved that the high uptake of (^{64}Cu, Mn)-FAP-PEG-MNPs in U87MG cells was due to the specific targeting of FAPI. In vivo pharmacokinetic test results showed that the pharmacokinetic formula of (^{64}Cu, Mn)-FAP-PEG-MNPs was as follows: %ID/g = 0.4665 + $43.7779*exp^{-80.15t} + 14.0071*exp^{-0.365t}$, the biological half-lives of drug distribution phase and scavenging phase were 0.086 h and 1.907 h, respectively. In U87MG tumor-bearing mice, MRI Imaging showed a clear increase in the T_1-weighted signal intensity after injection of Mn-FAPI-PEG-MNPs at 24 h compared to that in the prescan. Micro-PET imaging and semi-quantitative analysis of the region of interest showed that the probes were concentrated in liver, spleen and tumor sites in U87MG tumor-bearing mice, while the uptake in tumor sites was significantly lower in the A549 tumor-bearing mice. Meanwhile, Micro-PET imaging showed that the radiation signal of (^{64}Cu, Mn)-FAP-PEG-MNPs in the U87MG tumor site increased gradually over time and remained in the tumor site for more than 48 h.

Conclusion: The (^{64}Cu, Mn)-FAP-PEG-MNPs were successfully applied to multimodal imaging in U87MG model with high FAP expression. which showed excellent imaging quality in U87MG tumor-bearing mice for further tumor-specific therapy.

P-212
Comparison of biodistribution according to administration route for the development of liposome therapeutics

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Objective: Macrophages play an important role in in vivo immune defense and act as specialized antigen presenting cells to control early invasion by non-specific phagocytosis between pathogens and antigens and to induce antigen-specific T cell responses. Clodronate-liposomes are used to investigate the role of macrophages in immune and non-immune defense mechanisms through targeted and macrophage clearance studies. In this study, the difference in biodistribution of liposome compounds according to the route of administration using clodronate-encapsulated-liposomes (CL) and vehicle-liposome (VL) was confirmed using labeled isotopes using the click reaction.

Methods: The lipids used for liposome preparation are DSPC, cholesterol, DSPE-PEG(2k), DSPE-PEG(2k)-DBCO (click reaction with 64Cu-azide). CL was encapsulated at a concentration of 20 mg/ml, and a liposome size of 100 nm in diameter was prepared. On the surface of the prepared liposome, mannose for targeting macrophages and a fluorescent dye were bound to confirm the target macrophages in vitro. The macrophage target of these liposomes and the removal of macrophages by clodronate were confirmed by in vitro fluorescence microscopy using M2 polarized macrophages and in vivo PET scans. In the in vivo study, normal mice were used to compare the distribution of liposomal compounds according to the route of administration.

Results: As a result of in vitro studies, it was confirmed that cell viability was decreased in CL treated with high concentration in M2-polarized macrophages compared to VL, and was concentration-dependent. As a result of the in vivo studies using normal mice, it was confirmed that the distribution of the liposome compound in vivo differs depending on the administration route (I.V. or I.P.). In the I.V. route image, it was confirmed that the uptake in the liver and spleen of the two liposome compounds was maintained high up to 8 hours, and in the imaging of VL, uptake in the blood pool was also observed. In the I.P. route images, the uptake of both liposomal compounds was confirmed in the lymph nodes and the spleen. In mice administered CL, uptake of lymph nodes, liver, and spleen was maintained or increased over time. On the other hand, mice administered with VL showed a tendency to decrease overall absorption in vivo.

Conclusions: In this study, it was possible to confirm the role of clodronate acting on macrophages through M2-macrophage studies and in vivo imaging of mice. In vitro test showed a decrease in macrophages according to the concentration of clodronate and spleen uptake was shown in in vivo imaging. This is a result that is reflected in the theory that phagocytosis of liposomes introduced into the living body occurs in the spleen when looking at the mechanisms of bisphosphonate drugs such as clodronate in previous studies. In the present study, the liposome size is limited to 100nm. Therefore, we plan to check the distribution in vivo according to the size of the liposome through additional experiments. Confirmation of biodistribution according to the administration route or size of liposomes is expected to play an important role in the development of macrophage-based disease therapeutics.

P-213
DOX-PEG-^{198}AuNPs-PEG-Tmab: multimodal radiobioconjugate for targeted radionuclide therapy of HER2-positive cancers

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Objectives: Gold nanoparticles (AuNPs) with unique properties, such as small size, high biocompatibility, low toxicity, and versatility

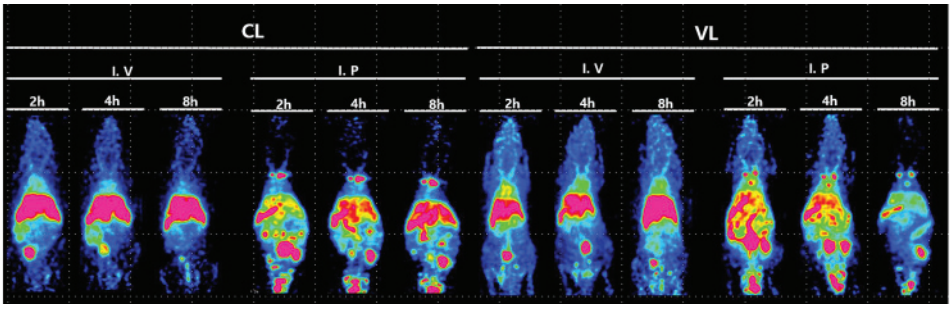

Fig. 1 (abstract P-212).

due to the ease of surface functionalization are very promising candidates for potential clinical ways of drug delivery [1,2]. The future of nanomedicine therapy lies in multifunctional nanoplatforms, which combine therapeutics compounds and targeting vectors – biomolecules specifically bind to the receptors of cancer cells. The aim of this study is the synthesis of novel multimodal radiobioconjugate containing simultaneously in one structure β^- emitter – ^{198}Au (^{198}AuNPs), a chemotherapeutic – doxorubicin (DOX) and a guiding vector – Trastuzumab (Tmab).

Methods: Based on Turkevich method [3], the synthesis of 30 nm gold nanoparticles using radioactive precursor (^{198}Au) was performed [4]. The size, zeta potential and shape of nanoparticles were determined by TEM (Transmission Electron Microscopy) and DLS (Dynamic Light Scattering) techniques. To stabilize nanoparticles, polyethylene glycol (PEG, 5000 kDa) was used. DOX and Tmab were attached to a bifunctional PEG linker comprising the thiol and carboxylic groups at both ends. To analyze obtained bioconjugates, DLS and UV-Vis methods were applied. To determine the effectiveness of synthesized radiobioconjugate *in vitro* studies such as receptor binding, internalization, MTS colorimetric test, apoptosis and cell cycle assay by flow cytometry, 3D cell culture (spheroids), confocal microscopy on two cancer cell lines, SKOV-3 (HER2+) and MDA-MB-231 (HER2-) were carried out.

Results: The DLS and TEM measurements confirmed the expected average size (~30 nm) and spherical shape. The zeta potential value showed high stability of ^{198}AuNPs without a tendency to agglomeration. The ^{198}AuNPs were successfully modified with bifunctional hydrophilic polymer PEG due to high chemical affinity of sulphur to gold. Subsequently, doxorubicin and Trastuzumab were attached to activated carboxylic groups of PEG leading to form irreversible peptide bond. The size and zeta potential values of obtained conjugates examined at each stage of synthesis, confirmed the formation of compounds. Initially, the cold compounds AuNPs, AuNPs-PEG-Tmab, Tmab, AuNPs-PEG-DOX, DOX, DOX-PEG-AuNPs-PEG-Tmab were tested and the cytotoxic effects of the last three were found. Successively performed cellular studies with the use of radioactive compounds indicated stronger effect of DOX-PEG-^{198}AuNPs-PEG-Tmab radiobioconjugate in comparison to ^{198}AuNPs and ^{198}AuNPs-PEG-DOX.

Conclusions: Targeted radionuclide therapy with DOX-PEG-^{198}AuNPs-PEG-Tmab is a promising approach for the treatment of HER2-positive cancers and warrants further investigation *in vivo*.

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Multimodality Imaging Probes

P-214
^{18}F-microbubble for PET/US imaging: conception and in vivo imaging

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Objectives: Molecular imaging is part of the standard care for many cancer types. Positron Emission Tomography (PET) is a gold standard for functional, metabolic and quantitative imaging of relevant biomarkers.[1] Ultrasound (US) is a broadly available, cost-effective and real-time imaging modality for anatomical and functional imaging for diagnosis. In addition, its mechanical ability to permeabilize cellular membranes is also expected to play a crucial role in therapy *via* drug delivery enhancement.[2,3] To leverage the best profit of both modalities to provide improved theranostic applications in oncology, lipid-shell microbubbles (μB) will be radiolabelled with fluorine-18. For this purpose, avidin-biotin coupling strategy was considered for microbubble radiolabelling.[4] The avidin is a glycoprotein with the capacity of binding four biotins. Taking advantage of its multivalent binding capacity, avidin will be used as an anchoring platform for: a^{18}F-radiolabeled biotin, a biotinylated μB and a biotinylated antibody (Ab) for specific tumour targeting.

Methods: In first place, a proof of concept was established using a biotinylated fluorophore (4-biotin-fluorescein). The 4-biotin-fluorescein was incubated with the avidin for 5 min in PBS, then, after steric exclusion chromatography (NAP-10), the resultant avidin-fluorescein complex was coupled to the biotinylated microbubbles (10 min in PBS) and finally the solution was analysed by microscopy (white light and λ_{ex} 475 nm). Once the proof of concept established, fluorine-18 radiolabelling of the biotinylated precursor was performed on a Trasis All In One module to obtain the desired radiotracer *via* nucleophilic substitution in DMSO at 160°C for 5 min using the K[^{18}F]F/K_{222} complex. The ^{18}F-biotin was coupled with avidin or avidin(Ab) in 1/1 ratio at room temperature in a saline solution. Then, the crude was purified by steric exclusion chromatography (NAP-10) in order to obtain the pure ^{18}F-biotin-avidin or ^{18}F-biotin-avidin(Ab) complexes.

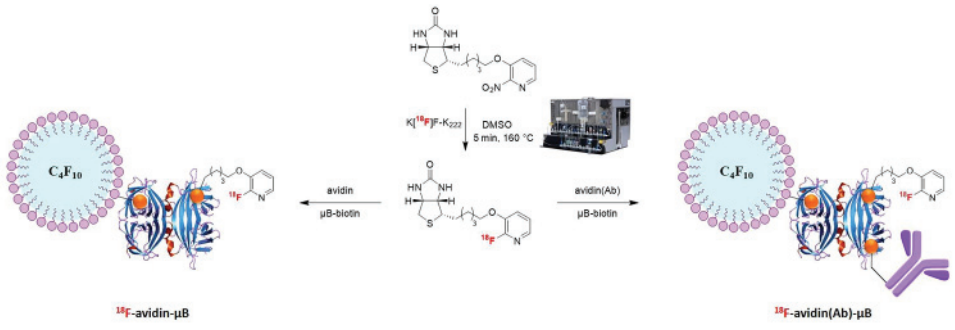

Fig. 1 (abstract P-214).