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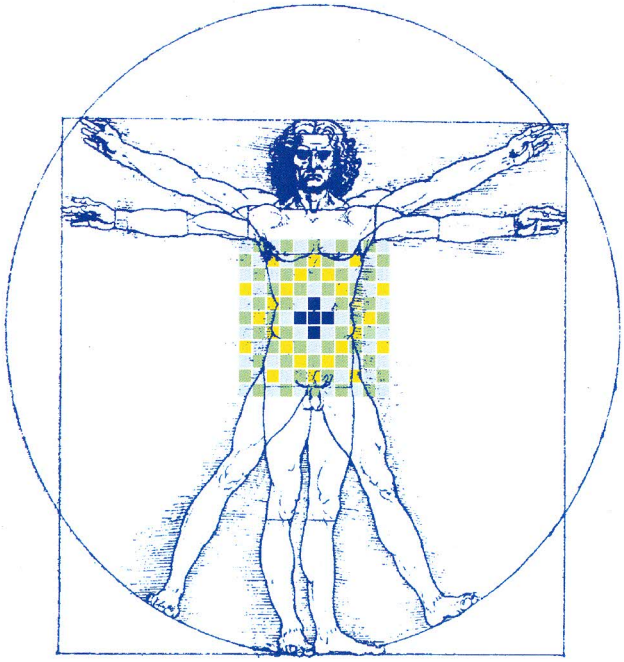
ABSTRACT BOOK

April 24-27, 2014
Pamplona, Spain



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tion in PET images (Figure 2). At this time point, the uptake ratios of testes/blood and testes/muscle were 7.30 and 4.80 respectively. For mice co-injected with Abiraterone, testes uptake was reduced (4.3% ID/g at 2h p.i.) and ratios of testis/blood and testis/muscle were 3.9 and 3.6 respectively. Significant *in vivo* defluorination was observed as bone uptake was 8.4 and 7.5%ID/g for unblocked and blocked study.

Conclusion. We successfully synthesized 3-[^{18}F]fluoroabiraterone, and performed imaging and biodistribution studies in male NODSCID mice. Using CYP17A1-expressing testes as surrogate of CYP17A1-expressing CRPC, good target-to-nontarget contrast images were obtained. Co-injection of Abiraterone reduced testes uptake, but not to background levels. Despite encouraging results, 3-[^{18}F] fluoroabiraterone is not recommended for CYP17A1 targeted imaging because of moderate non-specific binding as well as substantial *in vivo* defluorination.

OP07
Novel ^{111}In-estradiol based complexes for oestrogen receptor targeting

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Introduction. Imaging of oestrogen receptor (ER) status has been considered a useful strategy in the treatment planning and ultimate outcome of cancer patients since many tumour cells (breast, ovarian, endometrial) express high ER concentrations as compared to normal cells [1]. Additionally, it can help in the early prediction of the tumour responsiveness to hormonal therapy and in the treatment follow-up. Hence, the search for novel imaging agents to specifically target ER in tumours is an important but very demanding task that may benefit the selection of patients for individual therapy [2]. Herein, we describe the synthesis, characterization and biological evaluation of several ^{111}In-estradiol based complexes (Figure 1) stabilized with different bifunctional chelating agents (BFC) to access their feasibility for functional imaging of ER positive tumors.

Materials and Methods. New 16α-substituted estradiol derivatives bearing saturated/unsaturated spacer chains of variable length were conjugated to three BFC: DTPA, DOTA and DOTAGA [3,4]. The BFC-estradiol conjugates were then successfully coordinated with In. The relative binding affinity (RBA) of the new compounds to the human ERα and ERβ was evaluated by competitive radiometric binding assay. The ^{111}In complexes were obtained by reaction with $^{111}InCl_3$, at room temperature (DTPA like compounds) or 95°C, pH 5.0. Radiochemical purity and *in vitro* stability of the ^{111}In complexes were evaluated by thin layer and high performance liquid chromatography (ITLC and HPLC). The radiochemical stability was assessed in the presence of PBS and human blood serum, under physiological conditions and by exchange with apo-transferrin solution (3mg/mL, pH 7.5, 10mM sodium bicarbonate buffer), and DTPA solution (5 and 50 mM). Lipophilicity was determined through octanol/ PBS partition coefficient (Log $P_{o/w}$). Cellular uptake kinetics was assessed in suitable human breast cancer cell lines, such as MCF-7 (ER positive) and MDA-MB-231 (ER negative). Biodistribution and *in vivo* stability studies were also performed in immature female rats.

Results. ^{111}In estradiol based complexes were obtained at labelling efficiencies higher than 95% at low ligand concentrations typically after 10-20 minutes incubation. The structure of the radiolabelled complexes was assessed by HPLC comparison with the corresponding inactive In-estradiol complexes, fully characterized by ^{1}H-NMR, ^{13}C-NMR, IV, and electrospray mass spectrometry. The RBA results showed that estradiol-DOTAGA like compounds have high or moderate affinity to the ERα while having low affinity for the ERβ. ITLC and HPLC analysis indicated that all ^{111}In complexes, except one, are highly stable at 37° C up to 5 days in the presence of an excess of apo-transferrin and human blood serum. In the presence of an excess of DTPA solution, a low transchelation degree of the ^{111}In was observed. Log $P_{o/w}$ values were found to range from -0.99±0.02 to 0.43±0.03. Cellular studies indicate moderate uptake of the ^{111}In complexes in MCF-7 which decreases in the presence of estradiol. In MDA-MB-231 cells, the uptake is even lower than in the MCF-7 cells. Biodistribution studies indicated a rapid clearance from most organs, a long residence time into intestines and the hepatobiliar pathway as the main excretory route. HPLC analysis of urine and blood samples confirmed the high *in vivo* stability of the complexes.

Discussion and Conclusion. We have successfully synthesized and characterized new estradiol derivatives substituted at 16α position with different spacer chains and different bifunctional chelating agents. The estradiol-DOTAGA like chelators presented high or moderate affinity and selectivity to ERα. The ^{111}In-estradiol based complexes were prepared in high radiochemical yield and purity at low ligand concentrations. The complexes are kinetically stable *in vitro*, at 37° C, in the presence of the iron-transport protein, apotransferrin. Moreover, radioactive complexes do not undergo relevant transchelation in presence of DTPA and, except one, are stable in human blood serum. Animal studies indicate high *in vivo* stability and rapid clearance from main organs. The moderate cell uptake found is probably due to the low lipophilicity of the complexes. Nevertheless the relative ER binding affinity of the ^{111}In-DOTAGA-estradiol complexes and their evaluation in cancer cells suggest that uptake may occur via an ER-mediated process.

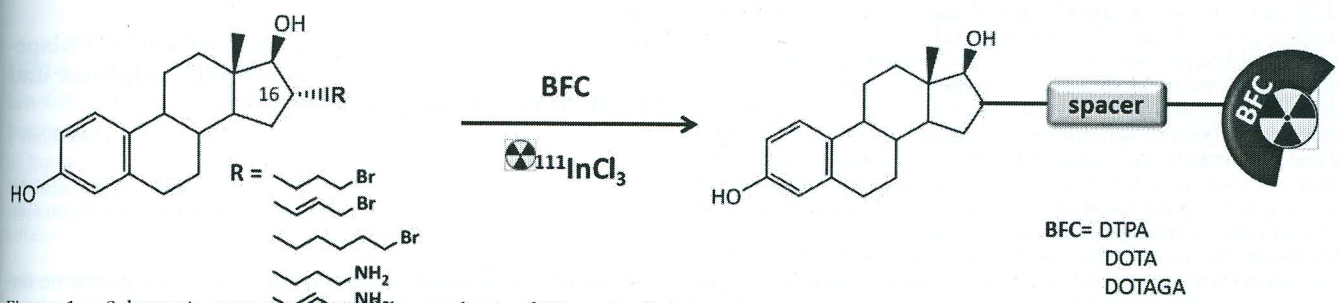

Figure 1.—Schematic representation of the synthesis of ^{111}In-estradiol complexes.

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APRIL 25, 2014

FRIDAY

LECTURE 2
(11:15-13:00)
Peptides and MoAbs

OP08
A new Methodology for Pretargeted PET Imaging: Click Chemistry at the Tumor

J. Lewis
New York

OP09
PET and SPECT imaging of rheumatoid arthritis with radiolabeled anti-FAP antibody correlates with severity of arthritis

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Introduction. Rheumatoid arthritis (RA) is an autoimmune disease that results in chronic and systemic inflammation in (synovial) joints. RA can be treated with a wide variety of drugs, ranging from anti-inflammatory drugs to disease-modifying anti-rheumatic drugs (DMARDs). However, the efficacy of many of these treatments is unknown and treatment schedules might be improved by using imaging techniques such as PET and SPECT imaging to adjust dosing, timing, etc. One of the dominant cell types which play a role in joint destruction are activated fibroblast-like synoviocytes in the synovial lining. It has been shown that these activated fibroblasts – similarly to those in tumor stroma – express fibroblast activation protein (FAP). Here we used an anti-FAP antibody, labeled with ^{89}Zr and ^{111}In to investigate the potential of specific imaging of FAP expression with PET and SPECT in an experimental mouse model of RA.
Methods. The monoclonal anti-FAP antibody 28H1 with high bivalent affinity for murine FAP (< 1 pM) and for human FAP (268 pM) was conjugated with desferoxamine using an activated TFP-ester, according to standard procedures. For ^{111}In labeling, 28H1 and the isotype control DP47GS were conjugated with isothiocyanate-benzyl-DTPA. Purified conjugated antibodies were labeled according to standard procedures. Radiochemical purity of the radiolabeled antibodies was higher than 99% as determined by ITLC.Arthritis was induced in DBA/1J mice by intradermal immunization at the tail base with 100 µg of bovine type II collagen (CII). On day 21, mice received an intraperitoneal booster injection of 100 µg of CII dissolved in PBS. The onset of arthritis occurred a few days after the booster injection. Macroscopic arthritis scores were based on the severity of the inflammation as well as on the number of inflamed joints. The severity of the inflammation ranged from mild to severe. Naive mice, i.e. not immunized, were used as control. All mice received an antibody dose of 50 µg (as determined in a previous dose optimization study) labeled with either 5-7 MBq ^{89}Zr for PET, 15-18 MBq ^{111}In for SPECT or 370 KBq ^{89}Zr or ^{111}In for biodistribution studies after dissection. Mice were scanned on a Siemens Inveon microPET/CT or a MIlabs USPECT-II microSPECT/CT scanner at 24 h and 72 h pi. All mice were euthanized at 72 h pi and tissues of interest were dissected, weighed and counted. In addition, separate groups of mice were injected with [^{18}F]FDG and were scanned and dissected 1 h pi.
Results. Both ^{89}Zr-28H1 and ^{111}In-28H1 showed high uptake in inflamed joints, which was 3-4 fold higher than that of the irrelevant isotype control DP47GS, strongly indicating specific accumulation of 28H1 in inflamed joints. Uptake of ^{111}In-28H1 ranged from 3.3 %ID/g in non-inflamed joints to 27.4 %ID/g in severely inflamed joints whereas control antibody DP47GS accumulation ranged from 2.5 %ID/g in non-inflamed tissue to 11.7 %ID/g in severely inflamed joints. Uptake of radiolabeled 28H1 in inflamed joints correlated with the arthritis score (r^2=0.92) and increased with the severity of inflammation, whereas the uptake of the control antibody remained low, also in severely inflamed joints (r^2=0.59). Blood levels of ^{111}In-28H1 at 72 h p.i. were 11.1 ± 1.9 %ID/g in control mice and were 4.7 ± 1.0 %ID/g in mice with severely inflamed joints. Uptake in all other organs was lower than the blood levels. Blood levels of DP47GS were generally higher than those of 28H1 (20.0 ± 4.9 %ID/g in control mice and 14.6 ± 4.7%ID/g in mice with severely inflamed joints). [^{18}F]FDG also accumulated in inflamed joints, but only in severely inflamed joints (max 11.5 %ID/g). [^{18}F]FDG uptake in the joints did not correlate with severity of inflammation (r^2=0.36). Both PET/CT and SPECT/CT imaging with radiolabeled anti-FAP antibody showed excellent delineation of the inflamed joints at both 24 and 72 h pi with both ^{89}Zr and ^{111}In labeled 28H1. Accumulation in non-target tissues was low, resulting in good target-to-background contrast. Contrast in [^{18}F]FDG PET/CT images was poor, mainly due to high background uptake.
Conclusions. The anti-FAP antibody 28H1 labeled with ^{89}Zr or ^{111}In showed excellent characteristics for imaging inflamed synovial tissue and uptake correlated with the severity of the inflammation. Inflamed sites could be clearly visualized by both PET/CT and SPECT/CT imaging.

OP10
Preclinical PET imaging studies of a novel bispecific heterodimer combining a PSMA inhibitor and a GRPR-targeting peptide

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Introduction. Radiometal-labeled peptidomimetic inhibitors targeting the prostate-specific membrane antigen (PSMA) have been shown to be clinically very attractive as they are effectively accu-