Experimental Details

Radiochemistry

Employing a fully automated system (GallElut^{+}, Scintomics GmbH, Germany) as described (1), non-processed eluate of a ^{68}Ge/^{68}Ga-generator with SnO_2 matrix (by IThemba LABS, SA; 1.25 mL, eluent: 1 M HCl, total ^{68}Ga activity 600–800 MBq) was adjusted to pH 2 by adding HEPES buffer (450 µL of a 2.7 M solution, prepared from 14.4 g HEPES and 12 mL water) and used for labeling of 0.3 nmol Aquibeprin or Avebetrin, respectively, for 5 min at 95 °C. Purification was done by passing the reaction mixture over a SepPak® C8 light solid phase extraction (SPE) cartridge, which was purged with water (10 mL) and the product eluted with an ethanol/water mixture (1:1 by volumes, 1 mL). Determination of radiochemical purity was done by Radio-TLC and Radio-HPLC as described before (*1*,*2*).

Cell lines and animal model

M21 human melanoma cells (*3*) were cultivated as described (*4*) in RPMI 1640 medium, supplemented with 10% FBS and 1% gentamicin (all from Biochrom AG, Berlin, Germany) at 37 °C in a humidified atmosphere containing 5% CO_2. Tumor xenografts were generated by injecting approx. 1.5×10^7 cells, suspended in serum-free medium supplemented with Matrigel® (Corning, #354262), into the right shoulder of 6–12 weeks old female SCID mice (CB17, Charles River, Germany). When tumors had grown to a diameter of 6–8 mm (usually 2–3 weeks after inoculation), anmials were subjected to PET studies or used for biodistribution. All animal experiments were approved by the local authorities and performed in accordance with current animal welfare regulations in Germany.

Biodistribution and PET studies

M21-bearing SCID mice, weighting 20–25 g at the time of final use for experiments, were administered 5–7 MBq of the radiopharmaceuticals for biodistribution, or approx. 20 MBq for PET, via tail vein catheters under isoflurane anaesthesia. After injections, animals were allowed to wake up with access to food and water.

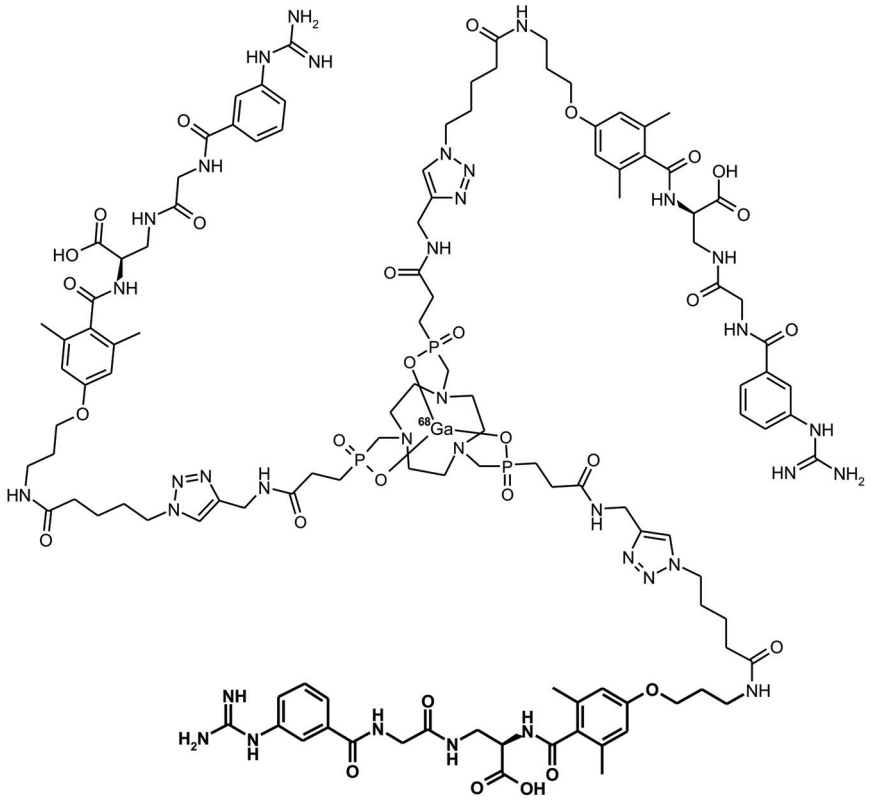
For biodistribution, animals were sacrificed after 90 min, organs harvested, weighed and the activity contained therein counted in a gamma-counter (Perkin-Elmer). For determination of ^{68}Ga-Aquibeprin uptake in the eye, only the eyeball without any adjacent tissue, such as the harderian gland, was excised. Calculation of injected dose per gram tissue was done from organ weights and counted activities, based on individually administered doses.

PET was recorded under isoflurane anaesthesia, 75 min p.i. for 20 min, on a Siemens Inveon small-animal PET system. Images were reconstructed as single frames using Siemens Inveon software, employing an ordered subset expectation maximum (OSEM) 3D algorithm without scatter and attenuation correction.

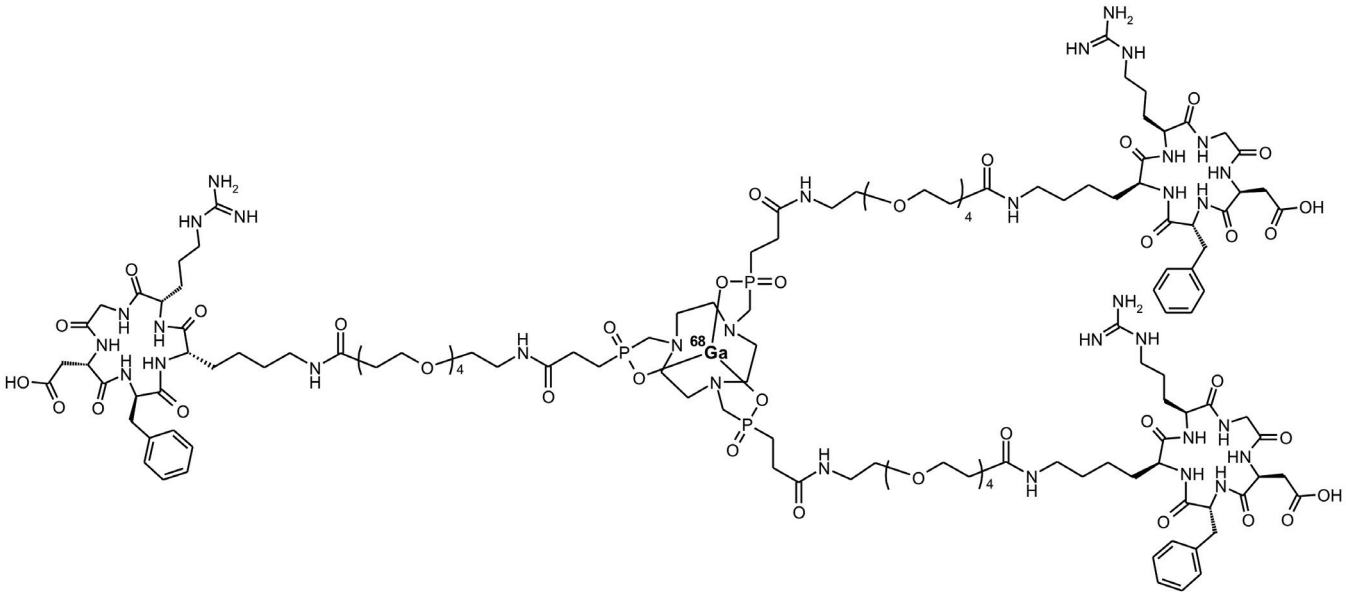
Immunohistochemistry

Tissues were fixed in 10% neutral-buffered formalin, routinely embedded in paraffin, and cut in 2 µm sections. After heat induced antigen retrieval (10 mM citrate buffer, pH 6), unspecific protein and peroxidase binding was blocked with 3% hydrogen peroxide and 3% normal goat serum (Abcam). Immunohistochemistry was performed with a Dako autostainer (DAKO) using antibodies against the $ß_3$ subunit (1:200, Abcam, 75872). For antibody detection, the Dako Envision-HRP rabbit labeled polymer (DAKO) was used, visualized by diaminobenzidine (DAB, Immunologic, BS04-500). Counterstaining was done using haematoxylin. Results were quantified by computer-assisted image analysis (Aperio Positive Pixel Count V. 9.1).

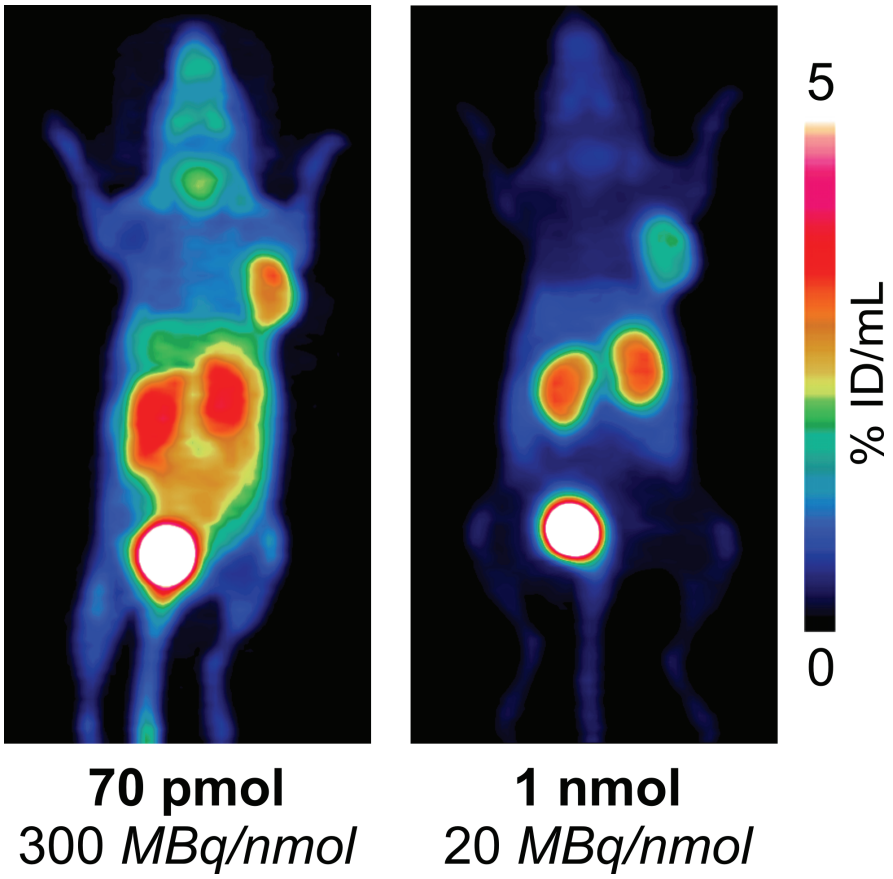
Additional Figures



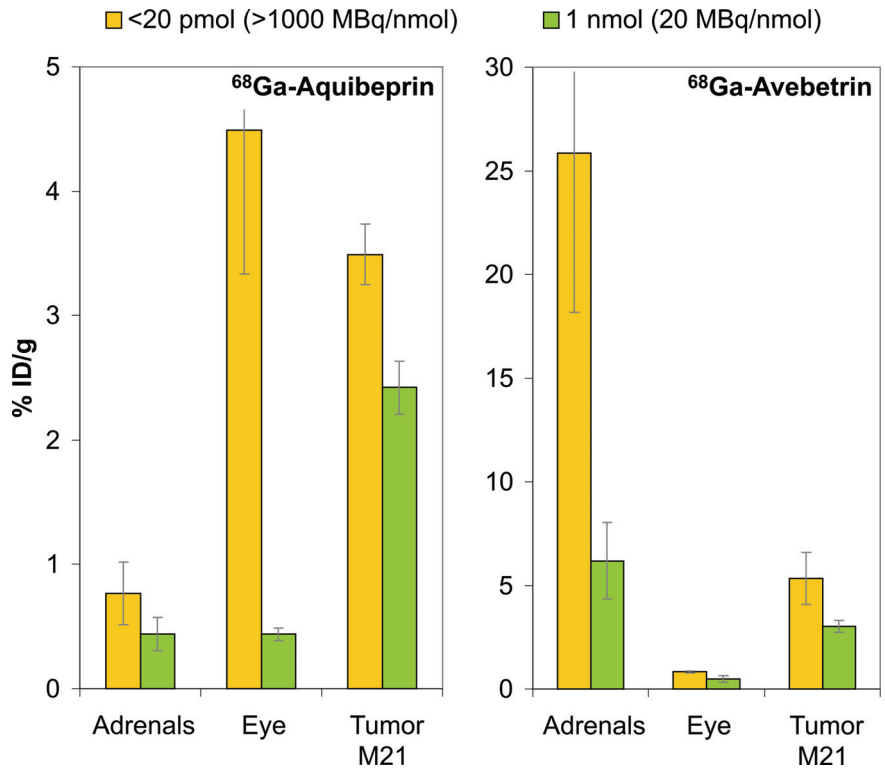
Supplemental Scheme 1: Structure of ^{68}Ga-Aquibeprin.



Supplemental Scheme 2: Structure of ^{68}Ga-Avebetrin.



Supplemental Figure 1: ^{68}Ga-Avebetrin PET images (MIP, 75 min p.i.) of the same M21 xenografted SCID mouse.



Supplemental Figure 2: Comparison of ^{68}Ga-Aquibeprin / ^{68}Ga-Avebetrin biodistribution in adrenals, eyes and M21 tumor, for high and intermediate A_S.

References

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2 Notni J, Steiger K, Hoffmann F, Reich D, Kapp TG, Rechenmacher F, Neubauer S, Kessler H, Wester HJ. Complementary, Selective PET-Imaging of Integrin Subtypes α5β1 and αvβ3 Using Ga-68-Aquibeprin and Ga-68-Avebetrin. J Nucl Med. 2016, DOI:10.2967/jnumed.115.165720.

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