Supplementary Figures

^{177}Lu-EC0800 Combined with the Antifolate Pemetrexed: Preclinical Pilot Study of Folate Receptor Targeted Radionuclide Tumor Therapy

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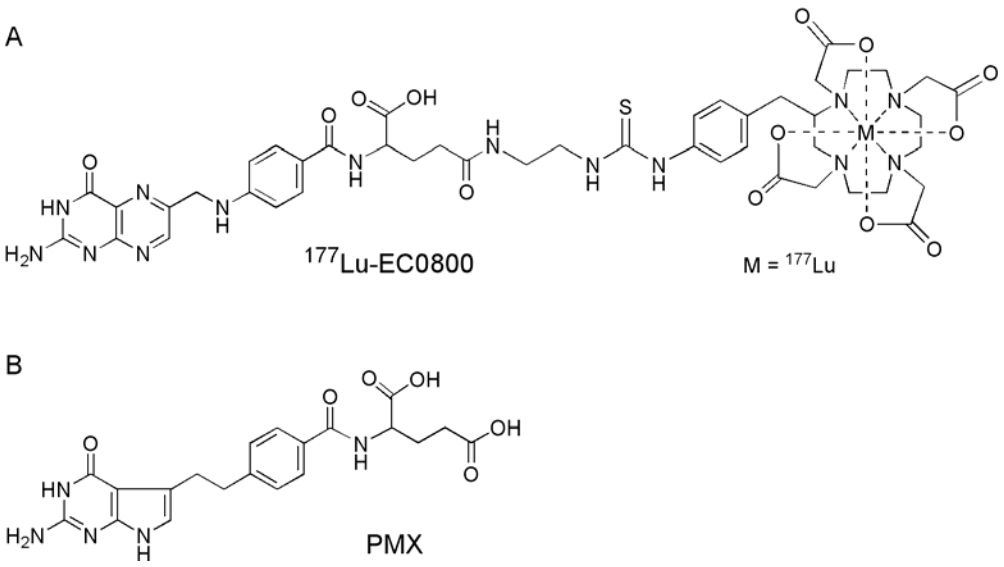
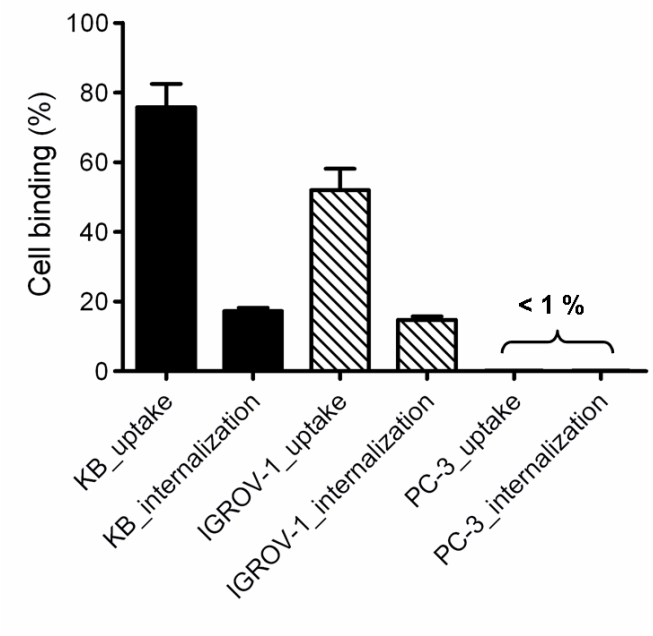
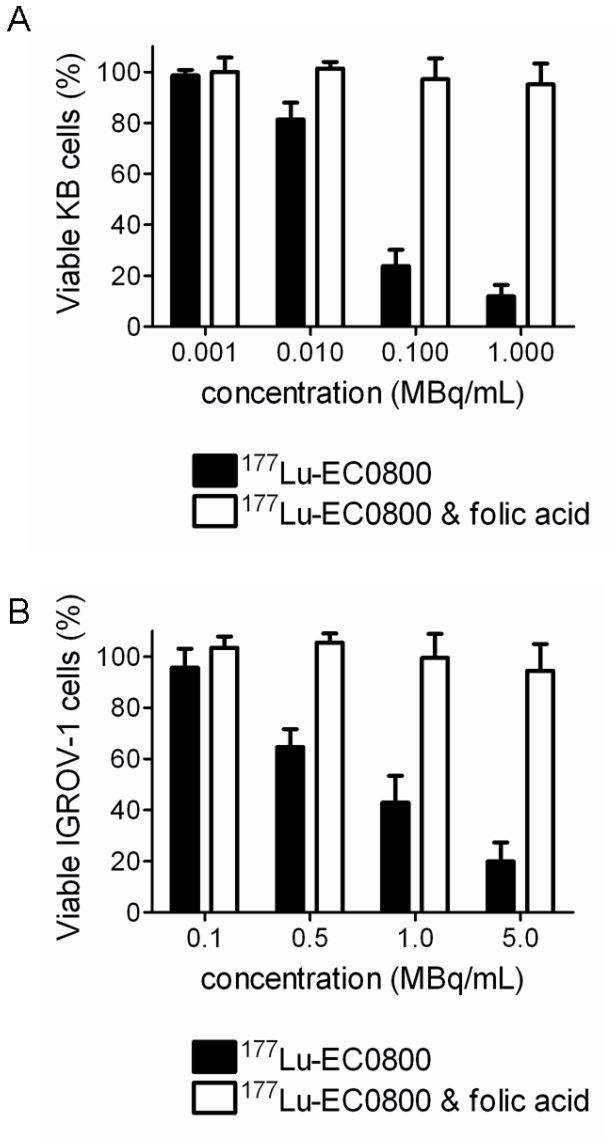


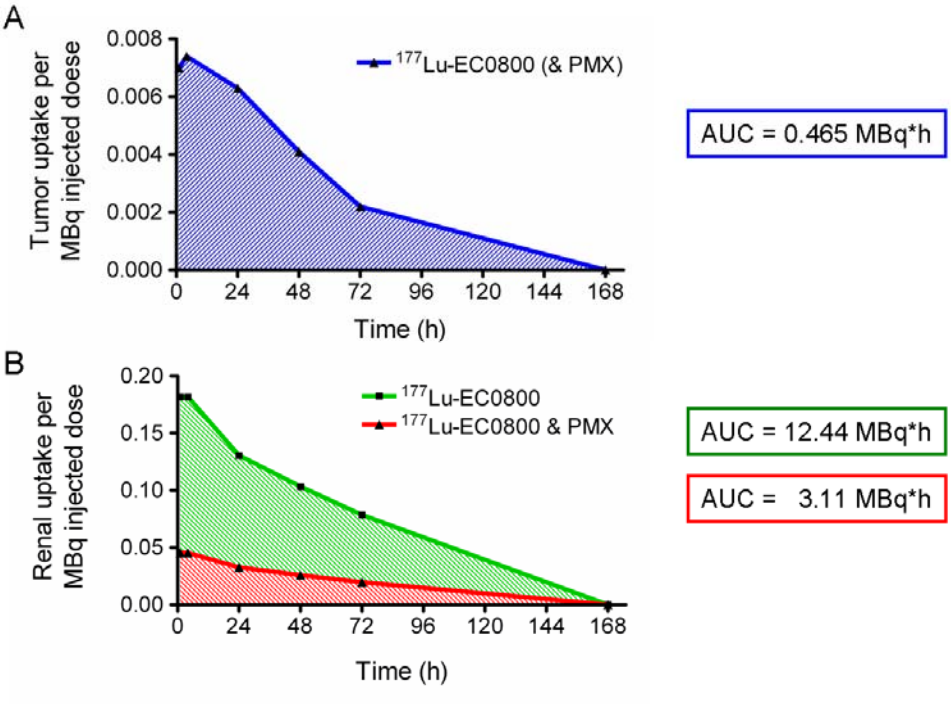
Supplementary Figure S1. Chemical structures of ^{177}Lu-EC0800 (A) and PMX (B).



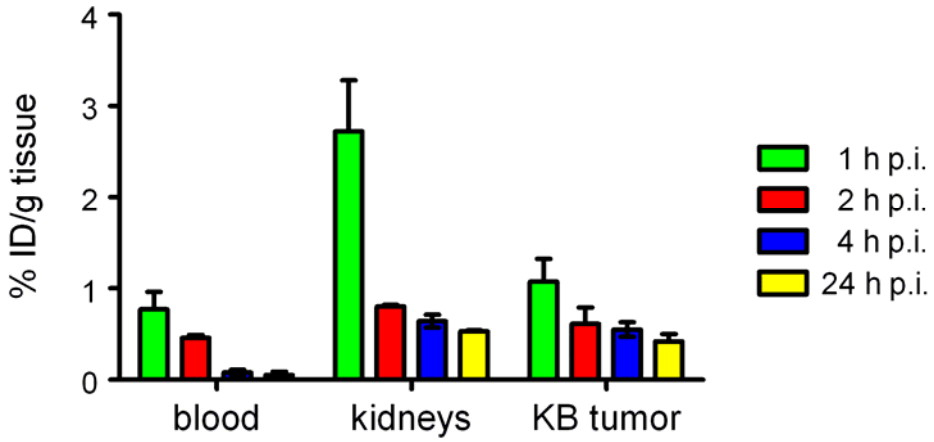
Supplementary Figure S2. Results of cell uptake and internalization studies performed with two FR-positive tumor cell lines (KB and IGROV-1) and one FR-negative tumor cell line (PC-3). Uptake of ^{177}Lu-EC0800 was observed in all FR-positive tumor cell lines. About 25-30% of FR-bound ^{177}Lu-EC0800 were internalized. In FR-negative tumor cells uptake/internalization of ^{177}Lu-EC0800 was not observed.



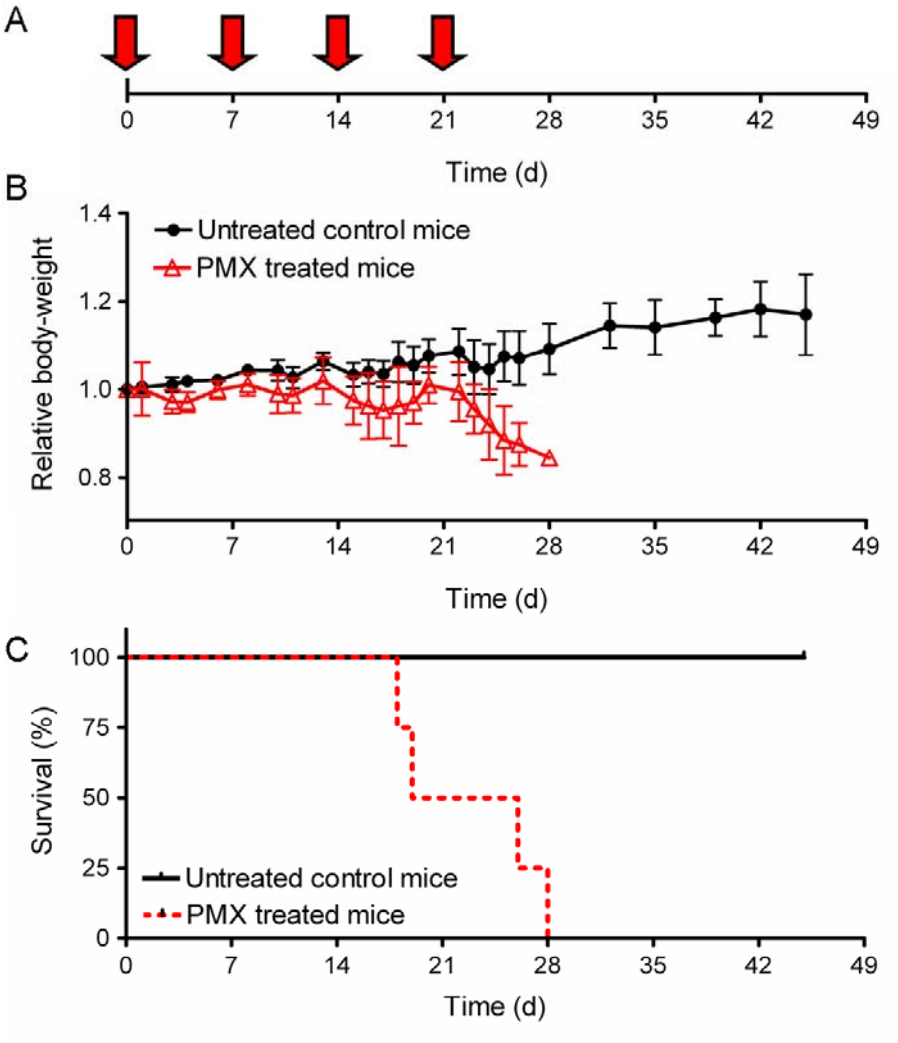
Supplementary Figure S3. A, clonogenic potential of KB cells and B, IGROV-1 cells upon exposure to increasing concentrations of ^{177}Lu-EC0800 with and without co-incubation of excess folic acid (100 μmol/L).



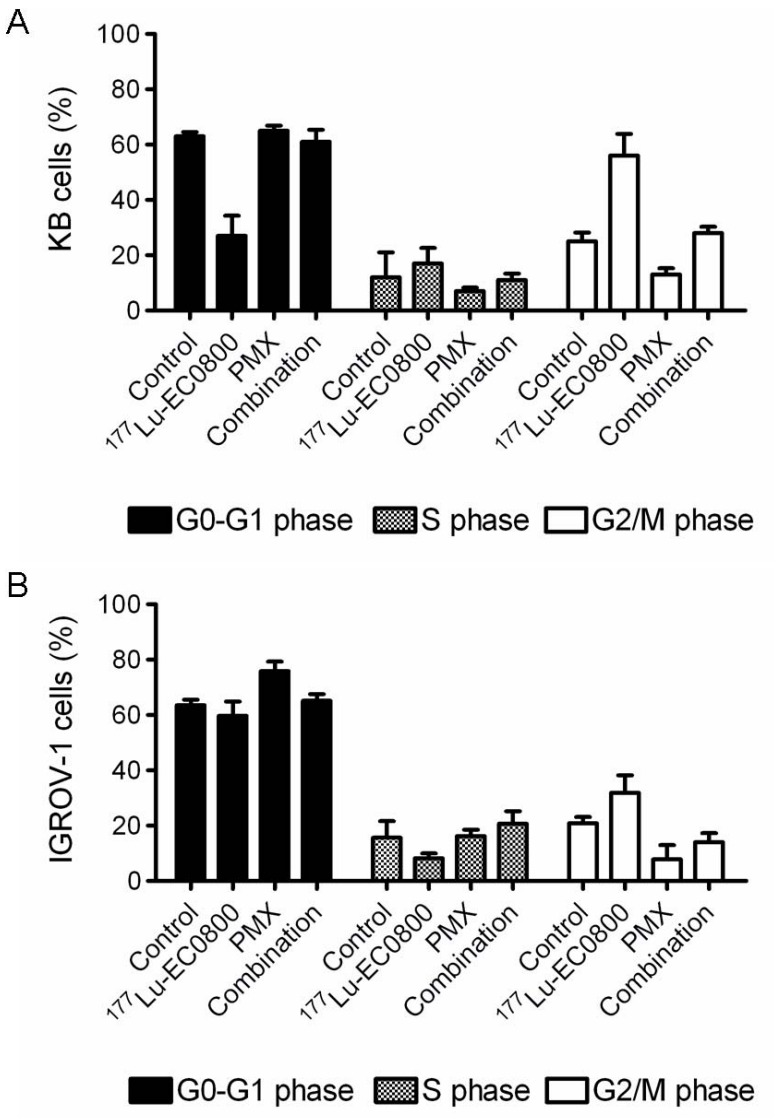
Supplementary Figure S4. Determination of the AUCs obtained from biodistribution data up to 72 h p.i. of ^{177}Lu-EC0800 in KB tumor-bearing mice under the assumption that radioactivity was completely excreted from tumors and kidneys 10 days after administration. A, AUC for the tumor tissue after injection of only ^{177}Lu-EC0800 (blue). B, AUC for the kidneys after injection of only ^{177}Lu-EC0800 (green) or in combination with PMX (red).



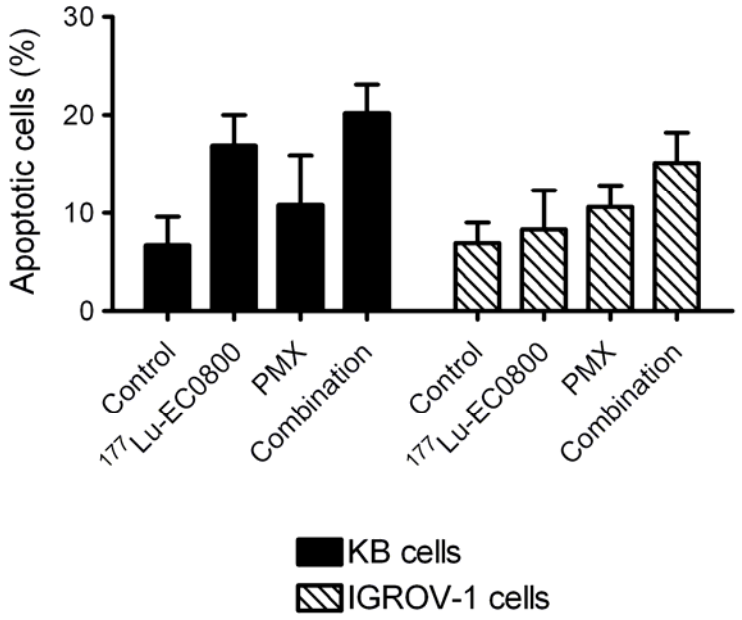
Supplementary Figure S5. Uptake of ^{3}H-PMX in blood, kidneys and KB tumor xenografts of nude mice at different time points after injection. PMX was cleared quickly from the blood circulation. At 1 h after injection of ^{3}H-PMX, retention of radioactivity in the kidneys was about 2.5-fold higher (~2.7% ID/g, 1 h p.i.) than in the tumor (~1.1% ID/g, 1 h p.i.). At later time points the retention of ^{3}H-PMX was comparable in tumors and kidneys was comparable.



Supplementary Figure S6. Determination of the maximal tolerated dose (MTD) of PMX in nude mice under the conditions of a folate-deficient diet. A, Injection scheme: 1 mg of PMX was intravenously injected at day 0, 7, 14 and 21. B, Graph of the average body weight of untreated control mice (n = 4) and mice treated with PMX (n = 4). C, Graph of the survival of untreated control mice and mice treated with PMX. It was found that two injections of 1 mg PMX was safe and hence the MTD was determined as 2 x 1 mg PMX. Reprinted (adapted) with permission from Müller et al. Mol Pharm 2013,10:967-74 (1). Copyright (2013) American Chemical Society.



Supplementary Figure S7. Cell cycle distribution of KB and IGROV-1 tumor cells was determined by flow cytometry upon exposure of these tumor cells to ^{177}Lu-EC0800 or to PMX as single agents and to the combination of these two agents. The tumor cells were incubated with ^{177}Lu-EC0800 and/or PMX for 4 h at 37°C followed by a drug-free period of additional 24 h before FACS-analysis. Control cells were processed equally but without addition of drugs to the cell culture medium.



Supplementary Figure S8. Upon exposure of tumor cells to ^{177}Lu-EC0800 or PMX as single agents and the combination of these two agents, fractions of viable cells, apoptotic cells and death cells were determined by flow cytometry. Incubation of the tumor cells with the drug(s) lasted for 4 h at 37°C followed by a drug-free period of additional 24 h before FACS-analysis. Control cells were processed equally but without addition of drugs to the cell culture medium. The graph shows the number of apopotic cells in percent of the total number of counted cells.

References

1. Müller C, Reber J, Schlup C, Leamon CP, Schibli R. In vitro and in vivo evaluation of an innocuous drug cocktail to improve the quality of folic acid targeted nuclear imaging in preclinical research. Mol Pharm. 2013;10:967-74.