Biological and clinical rationale for the development of CCK2-Gastrin receptor binding ligands for PRRT

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CCK2/Gastrin receptors: physiology

- Acid secretion, histamine and pepsinogen production (stomach)
- Neurotransmitter (Central Nervous system)
- Gall bladder contraction, intestinal motility (smooth muscle)
- Pancreatic islets and acini (secretion)

CCK2/Gastrin receptors: expression in tumors

	CCK1R	CCK2R/Gastrin-R
Neuroendocrine Tumors		
MTC	2/24 (8%)	22/24 (92%) 8/14 (57%)
SCLC	0/14 (0%)	7/32 (22%)
GEP	12/32 (38%)	4/28 (14%) 3/3 (100%)
Ovarian tumors		
epithelial	0/28 (0%)	
stromal	0/3 (0 %)	

Reubi, JC et al., Cancer Res 1997:57;1377-1386

Medullary Thyroid Cancer (MTC)

- Originates from parafollicular C cells of the thyroid, calcitonin (CT) secretion
- Rare cancer, 2-7% of all thyroid cancers
- ≈ 3000 cases per year in the US
- Surgery is only curative treatment

What causes MTC

- Sporadic (75%)
  - Somatic mutations of the RET proto-concogene in 25%
- Hereditary (25%)
  - Familial MTC or associated to MEN2 (A and B)
  - Activating germ line mutations of the RET proto-oncogene tyrosine kinase in 100% of cases

Biological features of MTC

- CCK2R expression
- Calcitonin secretion
  - Pentagastrin stimulation test
- CEA expression
- SSTR expression
- Mutations of RET tyrosine kinase

Clinical course of MTC

- Very variable
  - Good prognosis in young patients < 45yo
  - Poor prognosis in patients > 45 yo

- Prognostic indicators
  - CT doubling times
    - Poor – months
    - Favorable -- years

Where does it spread

- Lymph nodes in neck and mediastinum
- Lungs
- Bone
  - Often clinically occult
- Liver
  - Often clinically occult

Standard treatment after initial total thyroidectomy and LN dissection

- Localized metastatic disease
  - Surgery (neck, mediastinal nodes, bone)
  - External beam RT (bone mets)
- Diffuse disease
  - Liver, bone and lung mets
  - Standard chemotherapy unsatisfactory
  - No effective treatment

Possible new therapeutic targets

- CCK2R expression
  - That's what we're here for!
  - Development of new ligands for PRRT
- CEA expression
  - Pretargeted anti-CEA radioimmunotherapy
  - Improved survival in 29 pt series
    - JF Chatal, J Clin Oncol 2006:1705

Possible new therapeutic targets

- SSTR expression
  - Some reports of ^{90}Y-DOTATOC treatment
  - Not as satisfactory as with GEP tumors
- Protein kinase inhibitors
  - Clinical trials underway
    - Sorafenib, Suritinib
  - Evidence in animal models of synergy with standard chemotherapy

Radiolabeled DOTA-G-CCK8 for targeting CCK2 receptor expressing tumors

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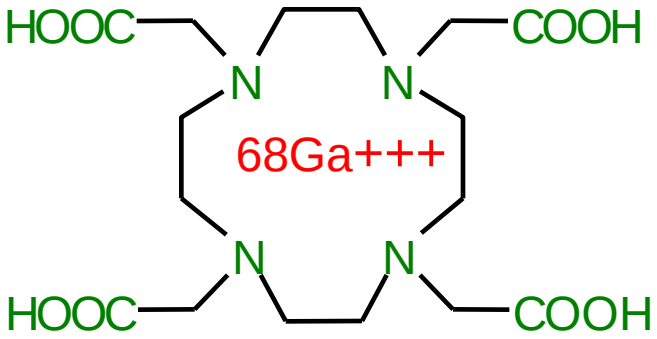
In vitro characterization: cell culture model

- CCK2R transfected cell
  - CMV promoter and neomycin resistance
  - NIH-3T3-CCK2R (Sue Watson, Nottingham)
  - A431-CCK2R
- Advantages
  - High levels of expression
  - Near constant levels of expression
  - Parental cell line is negative control



DOTA

PET Imaging

$^{90}Y/^{177}Lu$ labeling

G-CCK8:

Gly-Asp-Tyr-Met-Gly-Trp-Met-Asp-Phe-CO-NH_2

Short Minigastrin (MG11):

DGlu-Ala-Tyr-Gly-Trp-Met-Asp-Phe-CO-NH_2

Long Minigastrin (MG0):

DGlu-Glu-Glu-Glu-Glu-Glu-Ala-Tyr-Gly-Trp-Met-Asp-Phe-CO-NH_2

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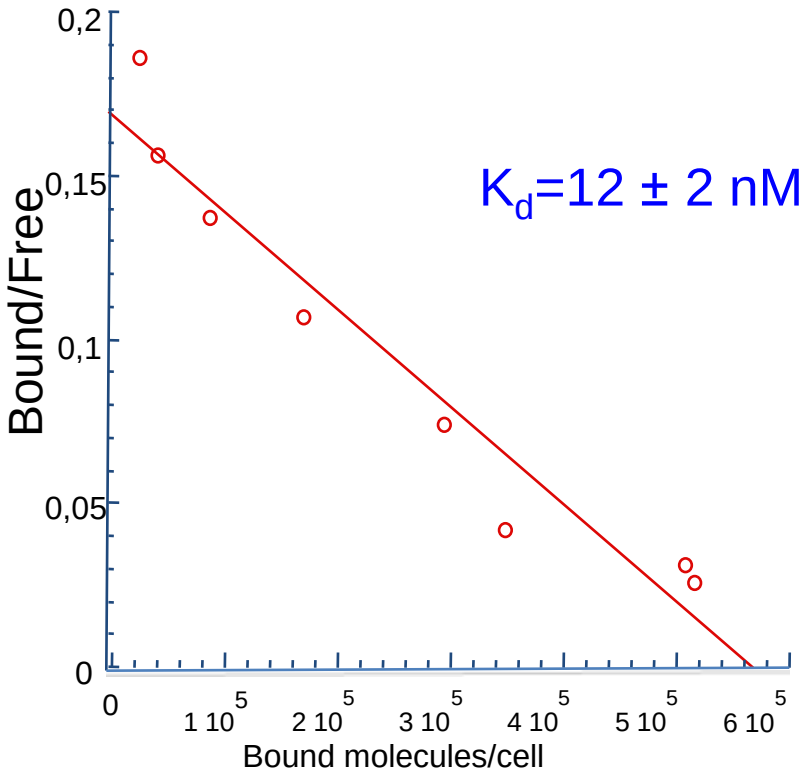
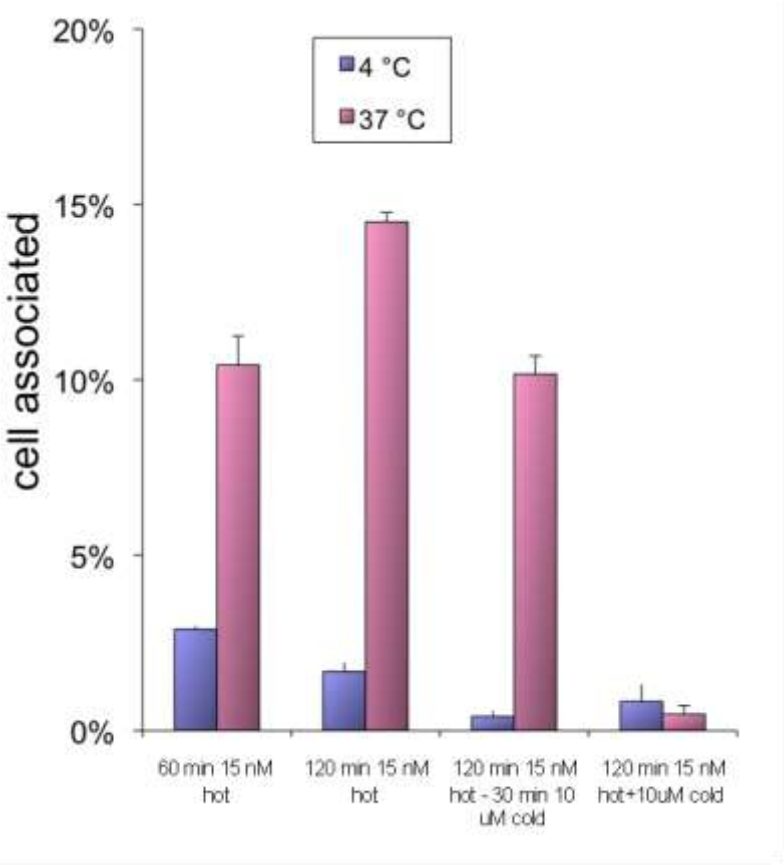
Binding Affinity CCK8 (A431-CCK2R)



K_d=12 ± 2 nM

Internalization CCK8



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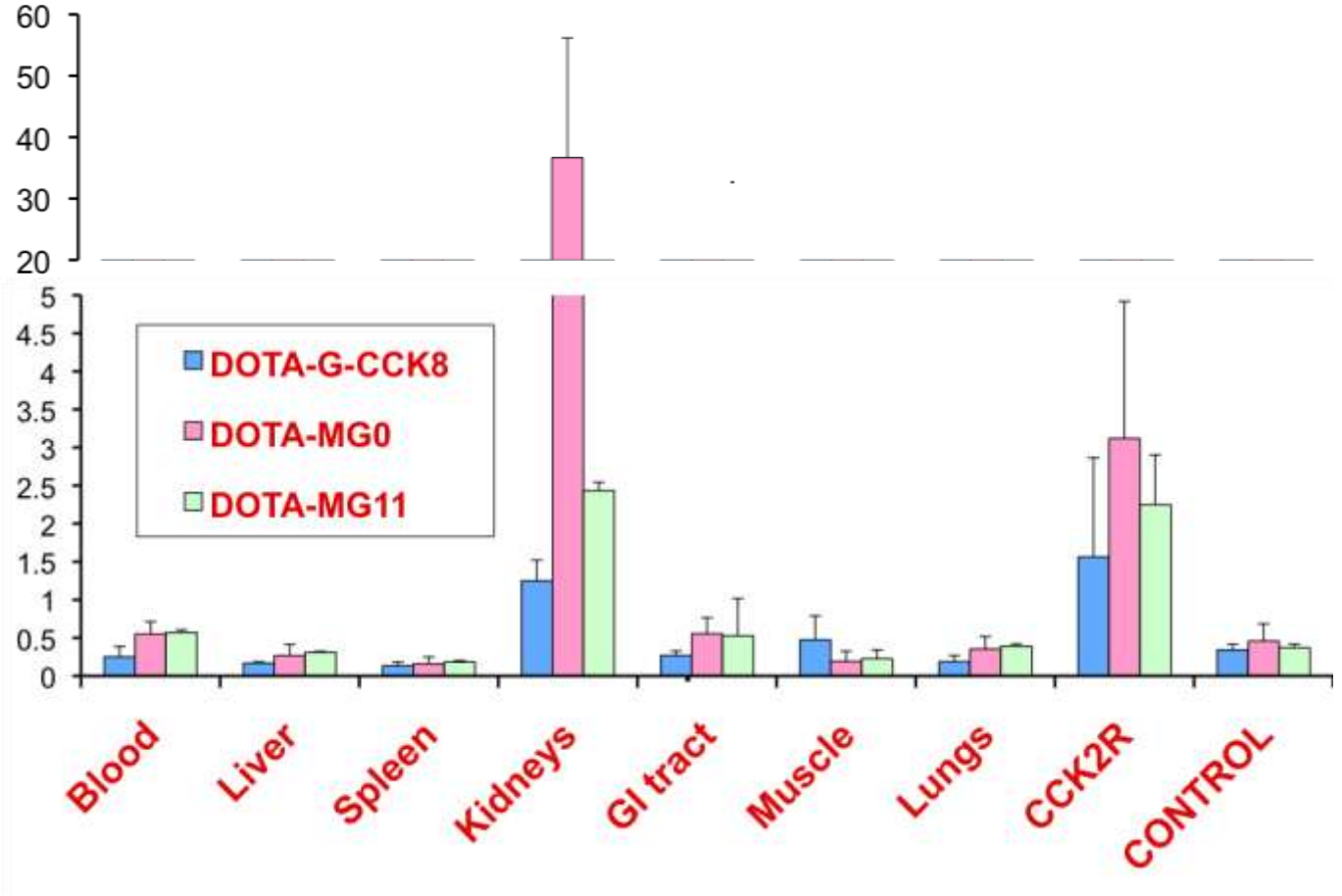
Biodistribution, 1h p.i.



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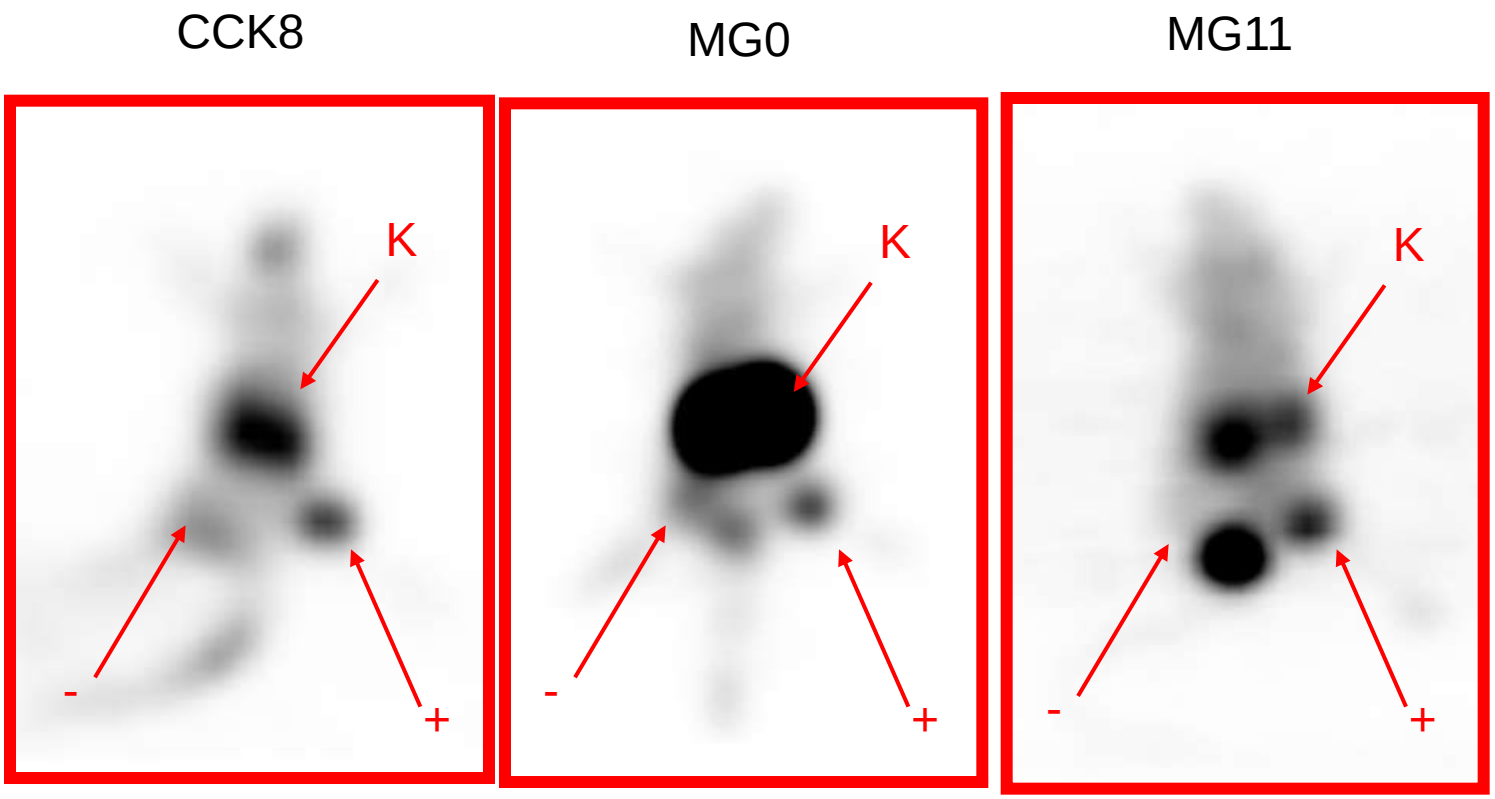
PET imaging, 1h p.i.



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Comparison at 1h p.i.

%ID/g	CCK8	MG0	MG11
Blood	0.25 ± 0.14	0.55 ± 0.17	0.57 ± 0.03
Kidneys	1.25 ± 0.27	36.66 ± 19.50	2.44 ± 0.11
CCK2R xenograft	1.56 ± 1.30	3.12 ± 1.80	2.25 ± 0.66
Control xenograft	0.34 ± 0.07	0.46 ± 0.23	0.37 ± 0.04

TUMOR TO BLOOD	1.2	0.1	0.9
TUMOR TO KIDNEY	6.2	5.7	3.9

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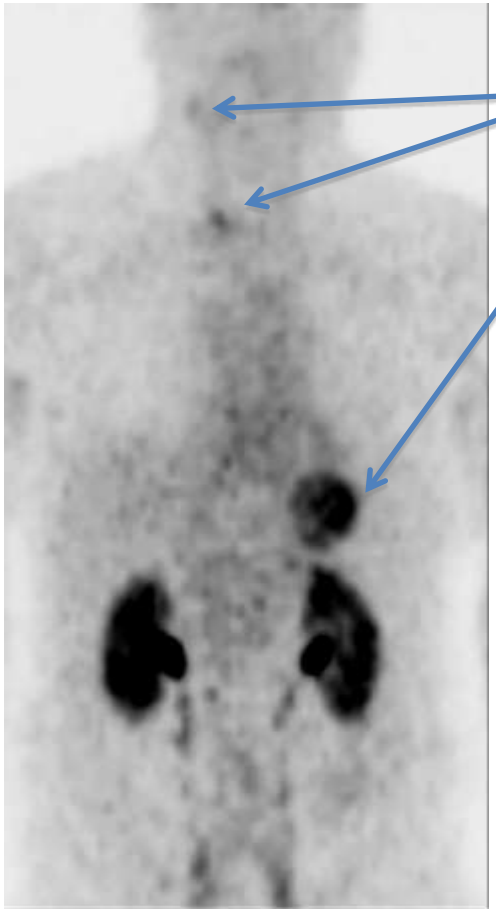
Whole body PET, 45 min p.i.



MTC LESIONS
AVG SUV 1.7-2

STOMACH
AVG SUV 4

^{68}Ga-DOTA-G-CCK8

For ^{90}Y ≈ ≈ ≈ 1 mGy/MBq

Dose to stomach wall in I131 therapy is ≈ 0.46 mGy/MBq (ICRP 53)

^{90}Y DOTATOC therapy
spleen ≈ 6 mGy/MBq
kidneys ≈ 3.5 mGy/MBq
liver ≈ 1 mGy/MBq

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Conclusions

- In vivo properties are very similar for the three compounds (labeling efficiency, binding affinity, etc.);
- MG11 has highest tumor uptake but marked kidney retention which make its use less desirable;
- CCK8 and MG0 show similar properties in vivo with CCK8 displaying better tumor/kidney and tumor/blood ratios;
- DOTA-G-CCK8 is our preferred candidate

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