Molecular Imaging of CXCR4 Receptors

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Hypoxic Areas of Tumors:
- CXCL12 expression by fibroblasts $\uparrow$
- CXCR4 expression on tumor cells $\uparrow$
- tumor cell motility $\uparrow$
- invasiveness $\uparrow$

CXCL12:
- promotes tumor cell growth by stimulating via CXCR4.
- induces recruitment of progenitors, which allow for tumor angiogenesis

CXCR4:
- activation of CXCR4 leads to targeted metastasis to the marrow or other sites of high CXCL12 expression. ("hijacking" of circulating tumor cells)
AIM:

to develop **suitable ligands** for CXCR4
for diagnosis, staging, therapy monitoring
Topochemical exploration of potent compounds using retro-enantiomer libraries of cyclic pentapeptides.

T140

FC131

Radioligand-Binding Studies (II)

Competitive binding curves of CPCR4 with $^{125}\text{I}$-CPCR4 and $^{125}\text{I}$-SDF-1$\alpha$ to Jurkat and CMS5/CXCR4 cells:

- Comparable IC$_{50}$ values were determined for CPCR4 at both cell lines with both radioligands

- $^{125}\text{I}$-CPCR4 shows very low non-specific binding
• High tracer accumulation in the CXCR4-expressing tumor
• Low background accumulation
• Higher tracer accumulation only in the metabolic and excreting organs (liver, intestine and kidneys)
Advanced Multimodal CXCR4-Tumor Imaging

Analysis of CXCR4/Luc expression and GFP control tumors:

- Photo
- MRI (mirror image)
- μ-PET
- Bio-luminescence
- GFP-Fluorescence

PET and Bioluminescence Imaging:

*In vivo* investigations of CXCR4 receptor status on tumors
Imaging CXCR4 Receptor Expression During Metastases

ex-vivo µ-Autoradiography: lung of a mouse 1h p.i. of nca. CPCR4 with a human SCLC (OH-1), primary tumor on the shoulder
Imaging of CXCR4 expression in lung metastases

μ-Autoradiography of lungs 1 h p.i. of $^{124}$I-CPCR4
Quantification of CPCR4-Binding ex-vivo

MicrolImager analysis of the lung of mice with s.c. growing metastasized SCLC tumor at 1h p.i.. Quantitative activity profile along an arbitrary selected direction.
CXCR4 expression in lung metastases: blockade

μ-Autoradiography of lungs 1 h p.i. of $^{124}$I-CPCR4 + xs CPCR4

$^{124}$I-CPCR4 + xs CPCR4
R = 6/1
Biodistribution $^{125}$I-CPCR4 2h p.i.

Biodistribution of $^{125}$I-CPCR4 in mice with OH-1 tumors 2 h p.i.
Acylated Orn-analogs – potential ligands for $^{18}$F-labelling

Derivatized cyclic pentapeptides:

Determination of $\log P$ value: 1.07

High lipophilicity of $[^{18}\text{F}]$-FB-OD25 will lead to high uptake in liver and intestine

Unfavourable biodistribution restricts use for future tumor imaging; further modification necessary.
Synthesis of cyclic pentapeptides with spacer at Orn

For $^{18}$F-labeling via aminoreactive prosthetic groups and simultaneous increase of hydrophilicity (e.g. PNA-linker):

IC$_{50}$: ~ 60 nM

IC$_{50}$: ~ 80 nM
Improved CPCR4 probe: $^{68}$Ga-DOTA-CPCR4-2.1

Biodistribution in mice with OH-1 tumors 2 h
$^{68}$Ga-CPCR4-2.1: imaging in OH-1 tumor bearing mice

**Animal 1:**

OH-1 SCLC, Injected with Ga-68-CPCRx (scale max: 177)

**Animal 2:**

OH-1 SCLC, Injected with Ga-68-CPCRx and xs cold peptide (scale: max 126)
Ga-68-labeled Ligands

<table>
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<tr>
<th>compd</th>
<th>n</th>
<th>IC_{50} [nM]^a</th>
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<tr>
<td></td>
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<td>a</td>
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<tr>
<td>29</td>
<td>6</td>
<td>1512.3</td>
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<tr>
<td>30</td>
<td>5</td>
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<td>31</td>
<td>2</td>
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<tr>
<td>32</td>
<td>1</td>
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<td>33</td>
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<tr>
<td>34</td>
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<td>334.7</td>
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<tr>
<td>35</td>
<td>-</td>
<td>903.05±439.75</td>
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<tr>
<td>36</td>
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<td>&gt;1000</td>
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<tr>
<td>37</td>
<td>-</td>
<td>807.5±477.4</td>
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^a IC_{50} values are given in nanomolar (nM).
Selection of tumor models to prepare first clinical studies
3D Surface Plot of Immunostained Section for CXCR4

rabbit UMB-2 monoclonal antibody has been analyzed using NIH Image Software. Distribution of the UMB-2 antibody around the membrane of positive OH-1 tumour cells. The intensity of the signal has been then plotted to create a 3D Surface Plot Image of the section.
Primary OH-1 Tumor Stained with UMB-2 Monoclonal Antibody
Dimeric DOTA labeled peptides

Demmer O. et al. (submitted)
# Dimeric DOTA labeled peptides

![Peptide Structure](image)

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<tr>
<th>Peptide</th>
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Dimeric DOTA labeled peptides

<table>
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<tr>
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<th>IC₅₀ [nM]</th>
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<td>92 ± 6</td>
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<td>0</td>
<td>In³⁺</td>
<td>29 ± 1</td>
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<tr>
<td>28</td>
<td>Gly</td>
<td>1</td>
<td>In³⁺</td>
<td>22 ± 5</td>
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Demmer O. et al. (submitted)
Dimeric DOTA Complexes

Brohl F. et al. (in progress)
A Triazacyclononane-Based Bifunctional Phosphinate Ligand for the Preparation of Multimeric 68Ga Tracers for Positron Emission Tomography

A Triazacyclononane-Based Bifunctional Phosphinate Ligand for the Preparation of Multimeric 68Ga Tracers for Positron Emission Tomography

Immunoimaging of CXCR4 expression in brain tumor xenografts using SPECT/CT
(Nimmagadda et al, J Nucl Med, 2009)

SPECT/CT of CXCR4 expression levels in experimental brain tumors using $^{125}$I-labeled anti-CXCR4 mAbs, hCXCR4 (12G5) and the control IgG$_{2A}$ MAb

The feasibility of the RID of CXCR4 expression imaging of the tumor microenvironment with a MAb is possible.

Biodistribution in U87 tumor-bearing SCID mice. 74 kBq of $^{125}$I-12G5 or $^{125}$I-IgG2A at 24, 48, and 72 h
U87-stb-CXCR4 mice (90 min p.i.)

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Receptors/cell</th>
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<tr>
<td>U87-stb-CXCR4</td>
<td>134,999 ± 20,341</td>
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<tr>
<td>U87</td>
<td>3,664 ± 802</td>
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<tr>
<td>DU4475</td>
<td>16,640 ± 5,128</td>
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<tr>
<td>MDA-MB-231</td>
<td>6,833 ± 1,570</td>
</tr>
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MDA-MB-231 (solid arrow) and DU4475 mice (open arrow)
Fluorescent Imaging of bladder cancer

Nishizawa et al., Int.J Cancer 2010

(Ac-Arg-Arg-Nal-Cys-Tyr-Cit-Arg-D-Lys*-Pro-Tyr-Arg-Cit-Cys-Arg-NH₂); D-Lys* indicates the carboxyfluorescein-labeled D-Lys
IC₅₀: 11nM

Figure 4. Representative microscopic view (a) and macroscopic view (b) of cut surfaces of mouse bladders. 0w: normal bladder, 12w: carcinoma in situ (CIS) caused by BBN. (c) After 12-week BBN drinking, multiple CIS lesions were observed by confocal laser microscopy with fluorescent images. Confocal laser microscopy of mouse bladder mucosa with phase-contrast image (upper column, ×100) and fluorescent image using TY14003 (lower column, ×100 and ×400). (d) Mouse bladders were directly observed under anesthesia by endoscope with white light images and fluorescent images. TY14003 instilled into bladder was illuminated through the bladder wall (right panel). (e) After the anterior bladder walls were cut and opened, bladder mucosa was washed twice and observed by white light (upper panels) and fluorescent light (lower panels). Fluorescent stain for TY14003 was observed in mice after 12-weeks BBN drinking (circle).
A CXCR4 antagonist CTCE-9908 inhibits primary tumor growth and metastasis of breast cancer
(Huang et al, Journal of Surgical Research, 2009)

Inhibition of CXCR4 reduced the primary tumor growth of MDA-MB-231 cells implanted into the inguinal mammary fat pad. (6 weeks)

In this model, 1·10⁵ MDA-231-BSC12 cells were injected into the left cardiac ventricle to produce bone metastases.

...provide an sensitive method to follow the progression of the primary and metastases over time