CAM-H2 effectively targets and treats HER2 positive brain lesions: a comparative preclinical study with Trastuzumab.

J. Puttemans[1], M. D'Huyvetter[1], A.D. Windhorst[2], F. van de Aa[2], T. Lahoutte[3], N. Devoogdt[1]

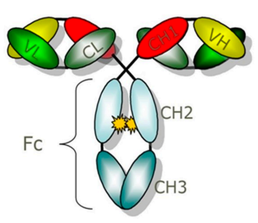
[1]In vivo cellular and molecular imaging Laboratory, Vrije Universiteit Brussel, BE; [2]Dept Radiology & Nuclear Medicine, Amsterdam UMC, location VUmc, Amsterdam, NL ; [3]Nuclear Medicine Department, UZ Brussel, BE

Contact: janik.puttemans@vub.be

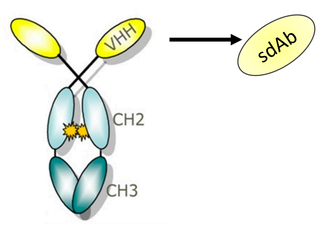
Introduction

Despite extensive research in systemic treatments for HER2 positive breast cancer brain metastases (BCBM), only limited benefits have been observed often at the cost of significant toxicities. Treatment failure is attributed to the limited capacity of drugs to cross the blood-tumor-barrier (BTB) or due to resistance mechanisms in the brain lesion microenvironment. In this study we evaluate the radiolabeled drug CAM-H2 as a new systemic treatment for the irradiation of HER2 positive BCBM.

Single domain antibody fragments (sdAbs) are small (15 kDa) heavy-chain-only antibody fragments. Due to their favorable pharmacokinetics, sdAbs are tested as targeting vehicles for the delivery of cytotoxic irradiation to cancer lesions inside and outside the brain. Here we measure the potential of CAM-H2 (131-Iodine conjugated anti-HER2 sdAb 2Rs15d) for targeting brain lesions with imaging and compare its therapeutic efficacy with Trastuzumab in a preclinical model.



Conventional antibody



Heavy-chain antibody and its derived Nanobody

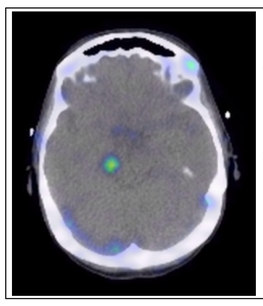
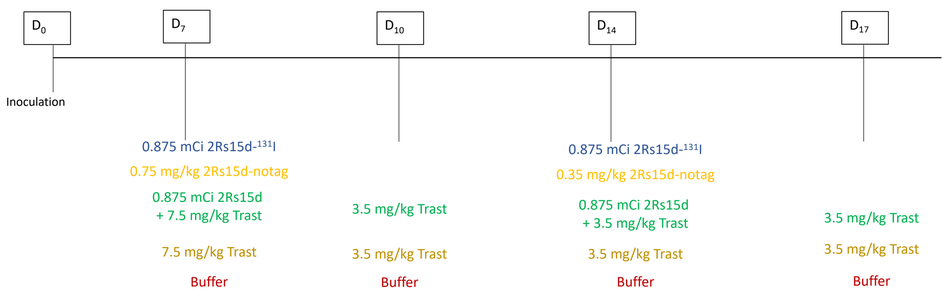


Figure: PET-CT scan of a HER2-positive breast cancer brain metastasis using ^{68}Ga-labeled anti-HER2 sdAb from Phase I clinical trial

Methods

Tumors were inoculated through intracranial injection of Fluc/HER2$^{+/+}$ SKOV3.ip1 or MDA-MB-231Br (MDA) cancer cells in the right frontocentral cortex. Tumor growth was confirmed using *in vivo* bioluminescent imaging. Biodistribution and tumor uptake of ^{111}In-radiolabeled 2Rs15d, Trastuzumab and a non-targeting sdAb R3B23 were evaluated through *in vivo* SPECT/CT imaging at 1h and 72h post injection followed by *ex vivo* tissue radioactivity counting. Five groups of MDA-tumor-bearing mice were treated with either CAM-H2, Trastuzumab plus CAM-H2, unlabeled 2Rs15d, Trastuzumab, or PBS (treatment regime left). Mice were sacrificed when weight loss exceeded 20% of the original bodyweight, or after occurrence of paralysis, neurological or behavioral abnormalities. The liver, spleen, kidneys, heart, brain were sampled, fixed in formalin and embedded in paraffin wax. Sections of 4 µm thickness were taken and stained with H&E. The sections were checked for quality and examined by light microscopy for signs of systemic toxicity.



Results

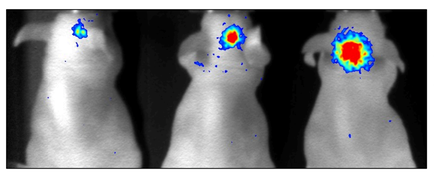


Figure 1: Follow-up of Fluc^{+} tumor cell growth with *in vivo* bioluminescent imaging 7 – 14 – 21 days post-inoculation.

SKOV3.ip1

2Rs15d-DTPA -^{111}In | **R3B23**-DTPA -^{111}In | **Trastuzumab**-DTPA -^{111}In

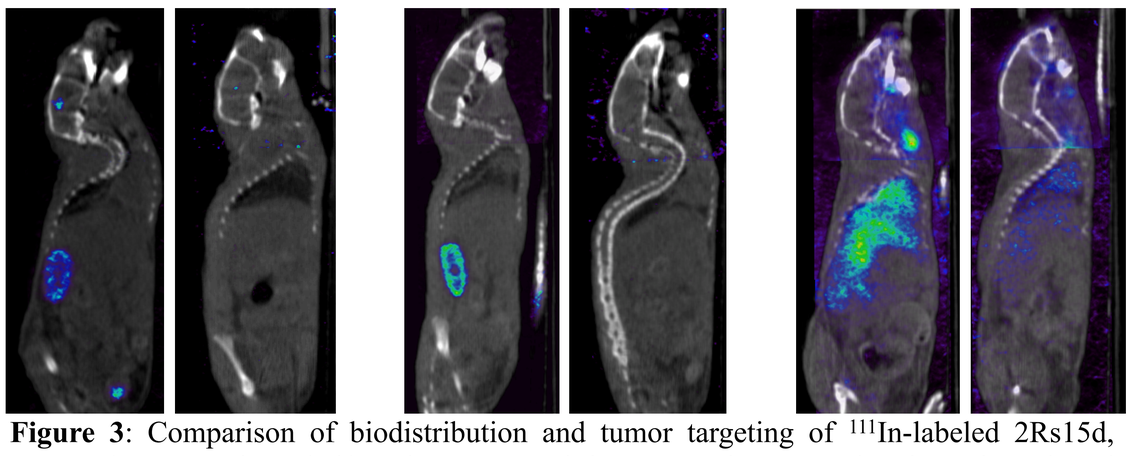


Figure 3: Comparison of biodistribution and tumor targeting of ^{111}In-labeled 2Rs15d, R3B23 (non-targeting sdAb) and Trastuzumab in SKOV3.ip1 tumor-bearing mice, 1h and 72h after tracer administration.

MDA-MB-231Br

2Rs15d-DTPA -^{111}In | **R3B23**-DTPA -^{111}In | **Trastuzumab**-DTPA -^{111}In

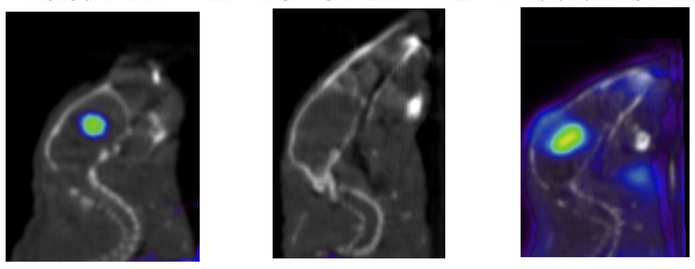
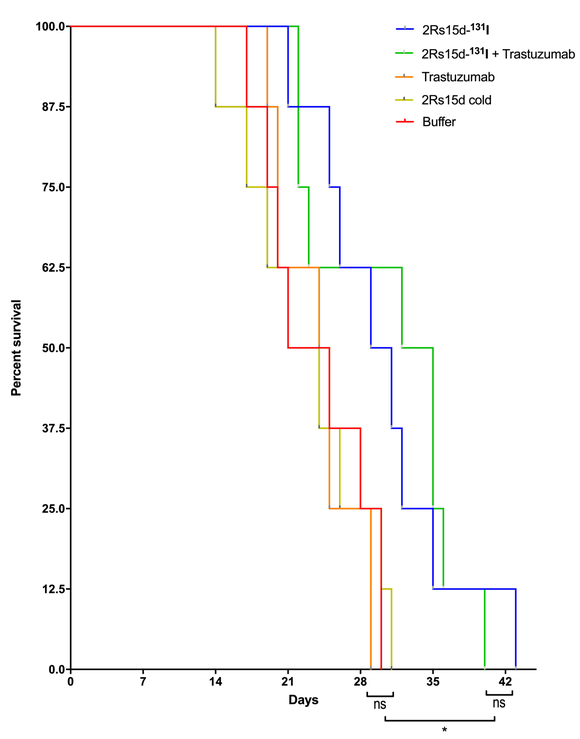


Figure 3: Comparison of tumor targeting of ^{111}In-labeled 2Rs15d, R3B23 (non-targeting sdAb) and Trastuzumab in MDA-MB-231Br tumor-bearing mice, 1h (sdAb) and 72h (mAb) after tracer administration.



Median Survival
- PBS: 23 days
- 2Rs15d cold: 24 days
- Trastuzumab: 24.5 days
- 2Rs15d – ^{131}I: 30 days
- 2Rs15d – ^{131}I + Trastuzumab 33.5 days

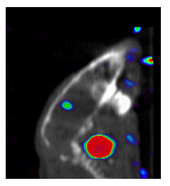


Figure 4: Mice treated with CAM-H2 and the combination of CAM-H2 plus Trastuzumab showed a significant increase ($p < 0.05$) in median survival compared to the Trastuzumab only, unlabeled 2Rs15d or PBS. The addition of Trastuzumab did not significantly prolong survival, compared to cold 2Rs15d or PBS. SPECT/CT imaging of CAM-H2 treated animals showed specific uptake in the brain lesions up to 2 days after administration.

Organ	Pathology	Severity
Kidneys	Multifocal tubular dilation	Minimal - Moderate
Heart	Inflammation, Myocardial necrosis	Minimal
Spleen	None	
Liver	Inflammation	None - minimal
Lungs	None	

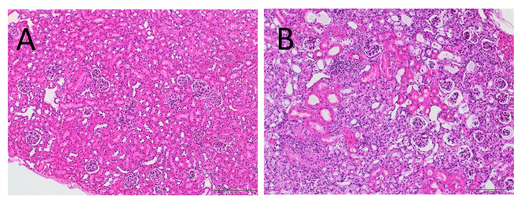


Figure 5: Overview of most common toxicity **A**. Kidney, PBS treated. No renal lesions. HE, lens x 10. **B**. Kidney, treatment with CAM-H2. Moderate tubular necrosis and regeneration with mixed-cell tubulointerstitial inflammation and interstitial fibrosis. HE, lens x 10

Conclusion

CAM-H2 crosses the BTB and binds HER2^{+} brain lesions. Treatment with therapeutic doses of CAM-H2 significantly increased survival compared to Trastuzumab. Systemically delivered cytotoxic irradiation conjugated to sdAbs has the potential to effectively treat cancer lesions inside the brain, with limited exposure to healthy brain. Clinical translation of ^{68}Ga-labeled 2Rs15d for diagnosis and CAM-H2 for treatment of BCBM is currently ongoing.

Conflict of Interest

M. D'Huyvetter holds ownership interest and is employee of Camel-IDS NV/SA. T. Lahoutte holds ownership interest and is consultant for Camel-IDS NV/SA. T. Lahoutte is member of the scientific advisory board of Ion Beam Applications (IBA) and member of the strategic committee of the Institute of RadioElements (IRE). N. Devoogdt holds ownership interest and is consultant for Camel-IDS NV/SA. T. Lahoutte and N. Devoogdt perform preclinical contract research for Roche and Telix Pharmaceuticals.

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