Establishing Micro Physiological Systems by means of a radiolabeled anti-EGFR antibody for the evaluation of new radioligands

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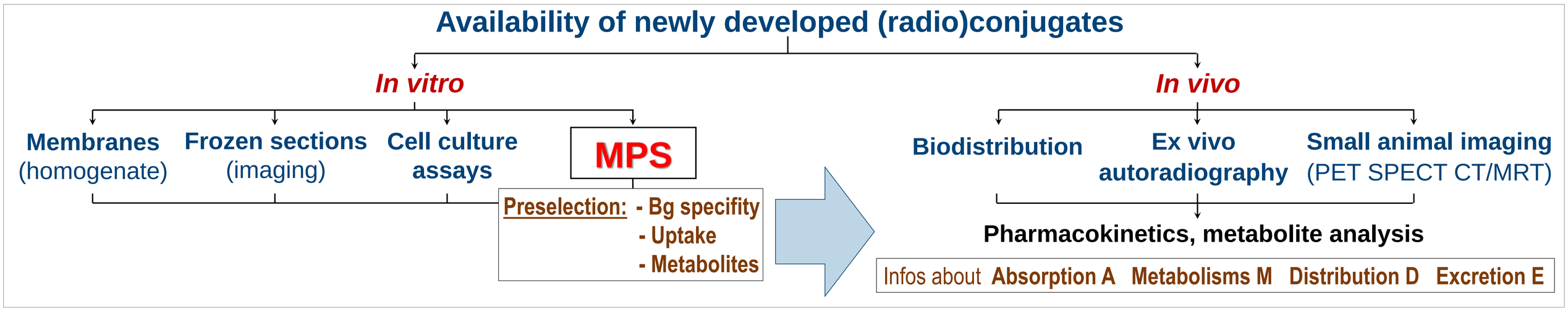
Motivation

Usual ⇨ **Small animal experiments** for evaluation of potential radiopharmaceuticals

Plan ⇨ **Reduction of animal experiments** by establishing an organ-on-chip technology

„Micro-Physiological Systems (MPS)“ [1,2]



Aim

Determination of pharmacological parameters such as **binding affinity (K_d) with MPS** on the example of radiolabeled anti-EGFR-antibody Cetuximab (C225)

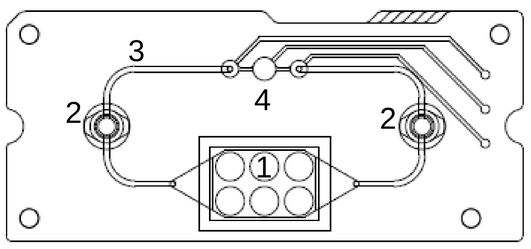
Conclusion

- **Binding affinity** of radiolabeled C225 feasible **with MPS**
- Low nonspecific binding on assay material

⇨ NEXT: **trials with kidney- and liver organoids using MPS**

Methods

Cell lines: - EGFR-positive **A431** (10^4) cultivated as monolayers or spheroids (0.8 ± 0.3 mm)
- EGFR-negative **MDA-MB435S** (10^4) as monolayer in the 6-well chamber of an MPS module (**Fig. 1**) for 24 h



1 – 6-well chamber
2 – Luer Locks for reservoirs
3 – circulation channel
4 – micropump

Fig. 1 Scheme of a MPS module

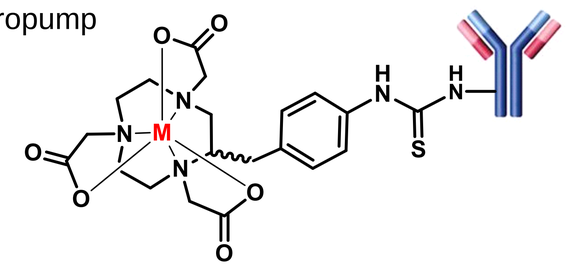
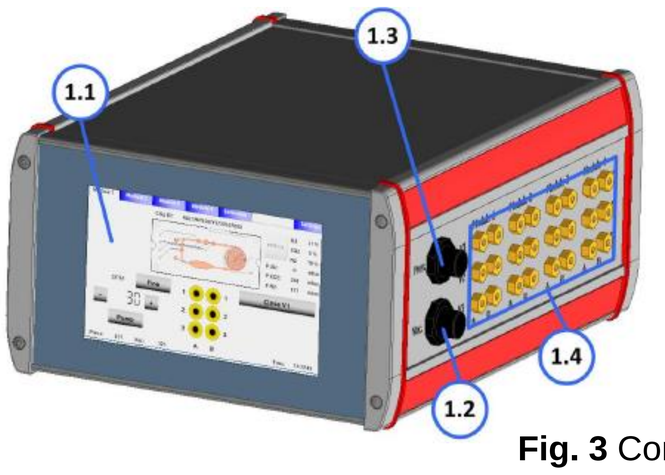


Fig. 2 NOTA-C225; **M: ^{64}Cu, ^{68}Ga**



1.1 – touchscreen display
1.2 – pressure control knob
1.3 – vacuum control knob
1.4 – pneumatic outputs

Fig. 3 Control unit, view: front/side

Assay: for preincubation pumping through the modul (**Fig. 3**) + medium (total binding) + medium with 0.8 µM ‚cold‘ C225 (nonspecific bg) + for 5 min at 80 bpm at a flow of 6.4 µL/s

for incubation + 1.2 to 15 nM radiolabeled C225 (**Fig. 2**) + in a total volume of 1 mL for 15 min + 10 min washing with PBS + 20 min exposing MPS modules to imaging plates (BAS, Fuji) + evaluation with AIDA (Elysia-Raytest) /GraphPad Prism

Results

Spheroide formation:
- **A431** cells incubated with biocompatible magnetic poly-L-lysine coated iron-oxid-gold-nanoparticles
- at least 5 h later sowing the cells in well plates and placing them over arranged button magnets
- spheroids usable after 24 hours (**Fig. 4**)

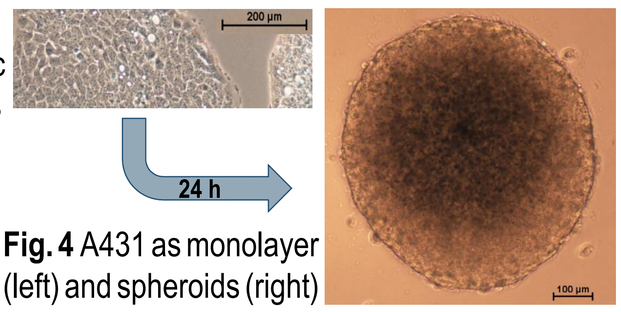


Fig. 4 A431 as monolayer (left) and spheroids (right)

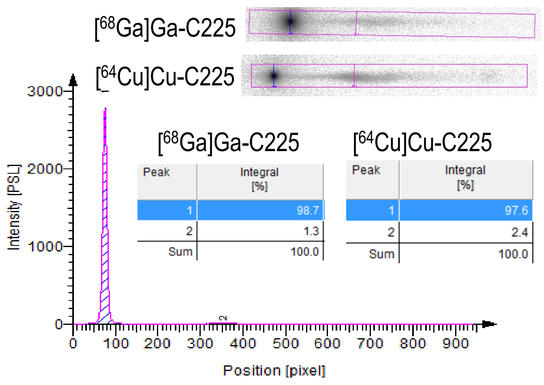


Fig. 5 Results TLC of labeled C225

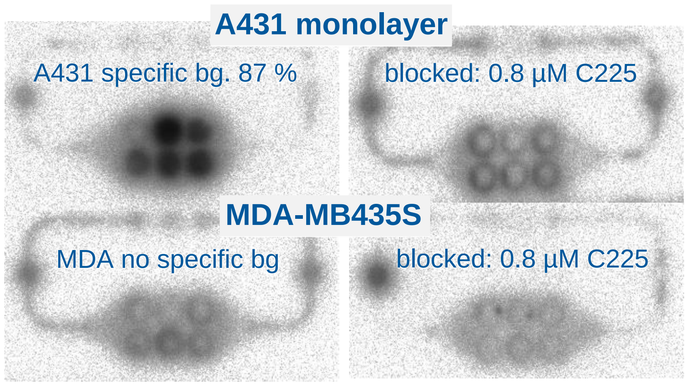


Fig. 6 [68Ga]Ga-C225 (2nM) in MPS

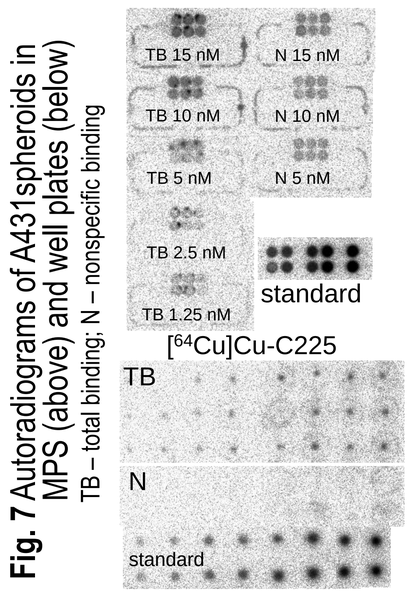


Fig. 7 Autoradiograms of A431spheroids in MPS (above) and well plates (below)
TB – total binding; N – nonspecific binding

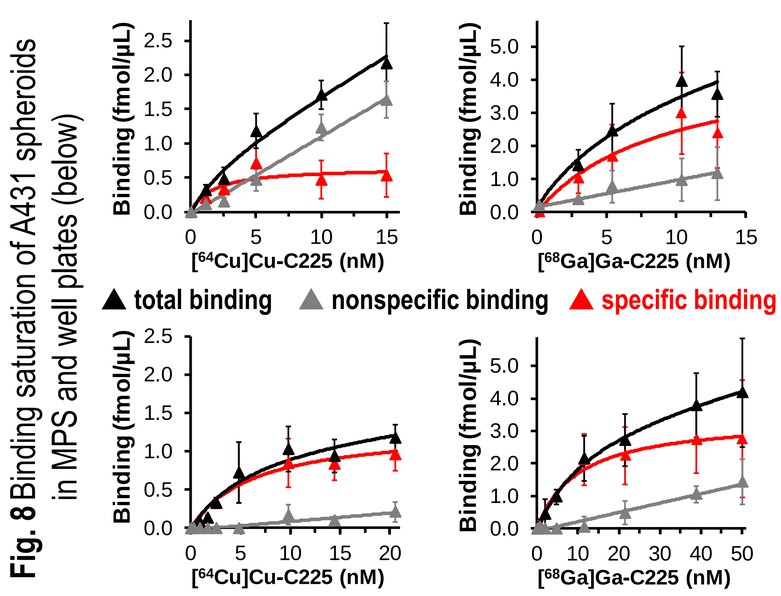


Fig. 8 Binding saturation of A431 spheroids in MPS and well plates (below)

Saturation K_d(nM)	[64Cu]Cu-C225	[68Ga]Ga-C225
Chip A431 spheroids / ML	9.5 ± 5.8 / 3.1 ± 0.7	9.4 ± 7.8 / 24.9
Well plate A431 spheroids	4.4 ± 2.1	10.2 ± 2.1
(ML – monolayer)	Mean ± SEM	

References

[1] Busek et al., J Sens Sens Syst 2016, 5, 228.
[2] Schmieder et al., Proc SPIE 2020, 11268, 1126804_1.

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