Nanobodies catch a tumor: red handed!!

Groep 5

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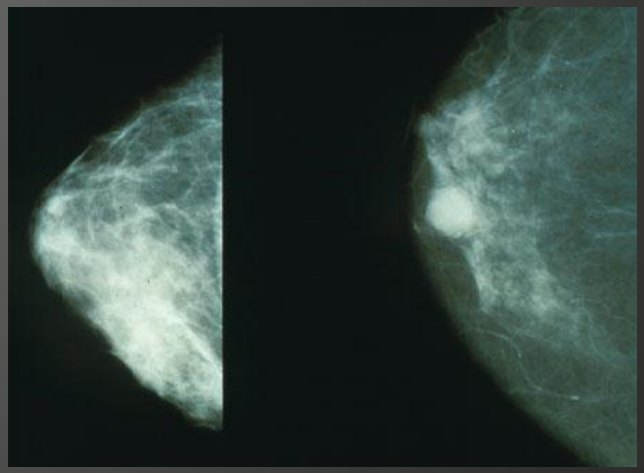
Today's speakers: Mark & Joren.

Outline

- Introduction
  - Breast cancer
  - HER receptors
  - Nanobodies
- Our research
- Relevance

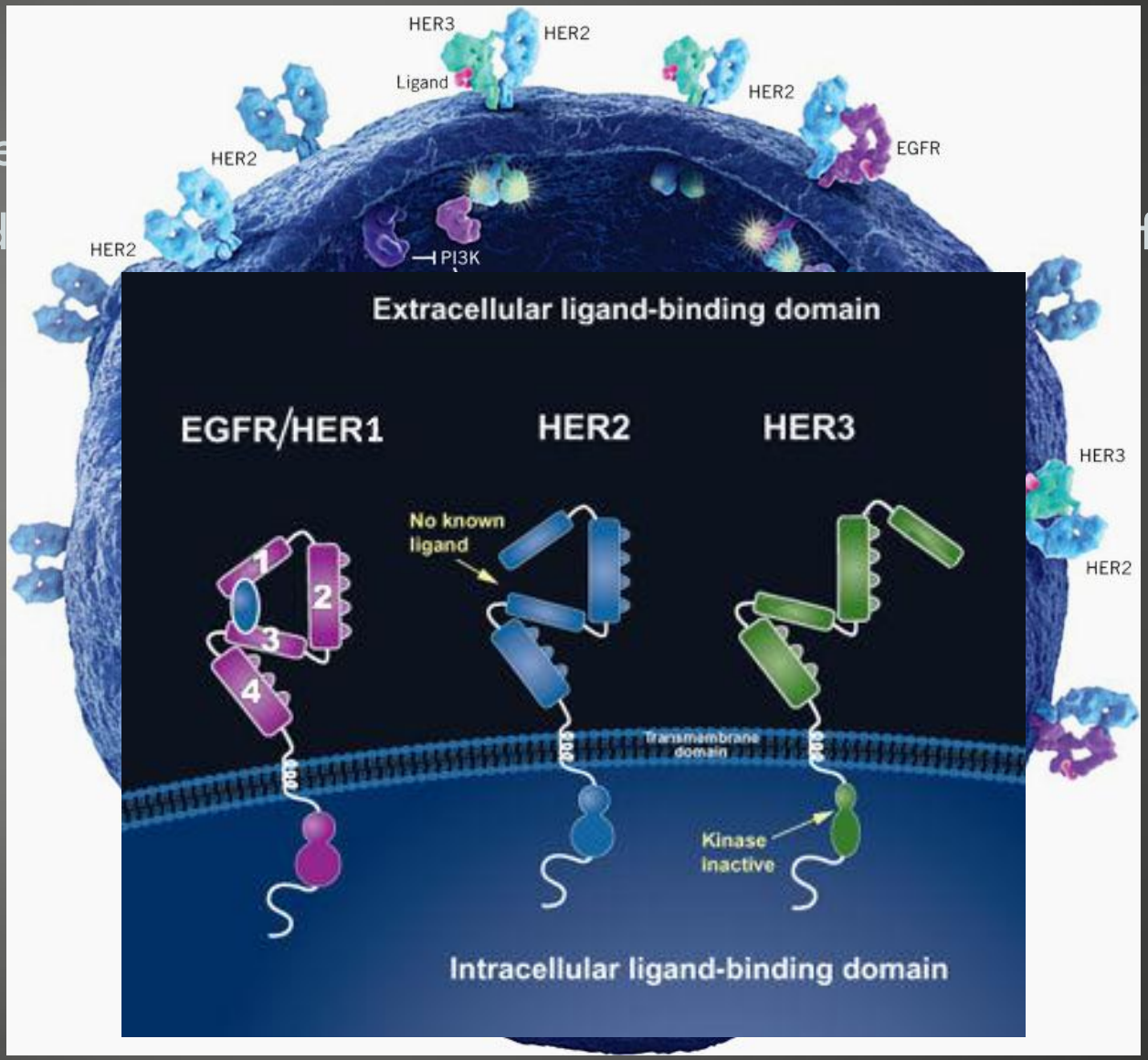
Breast cancer

- Yearly cancer takes many lives
  - Breast cancer is number 1 cause of death in women between 35 and 50!!
- Both men and women are affected
  - 30 men died of breast cancer in 2008
- Research
  - Earlier detection
  - Younger age
  - Nanobodies



HER 1/2/3/4

- Differe
- Ligand firmation



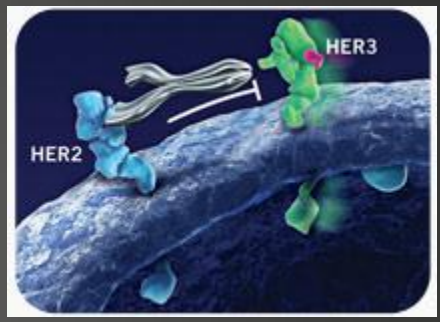
Human Epidermal Growth Receptors

30% of all breastcancers over express HER-2

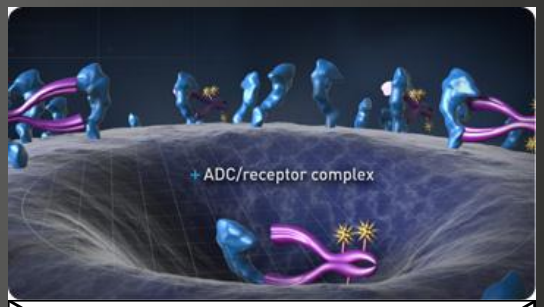
- Therapeutic options
  - Trastuzumab: multiple effects
  - Pertuzumab: prevents dimerization of HER-2
- Diagnostic options
  - Scans
  - More precise operation
  - Nanobodies

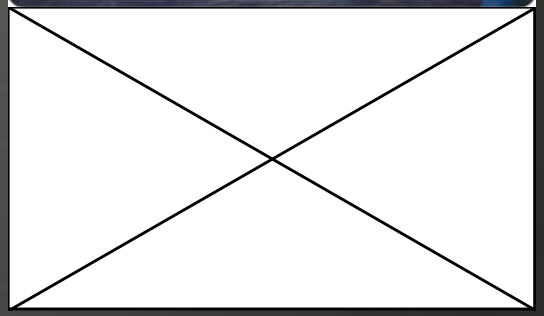
Pertuzumab

Dimerization blocking



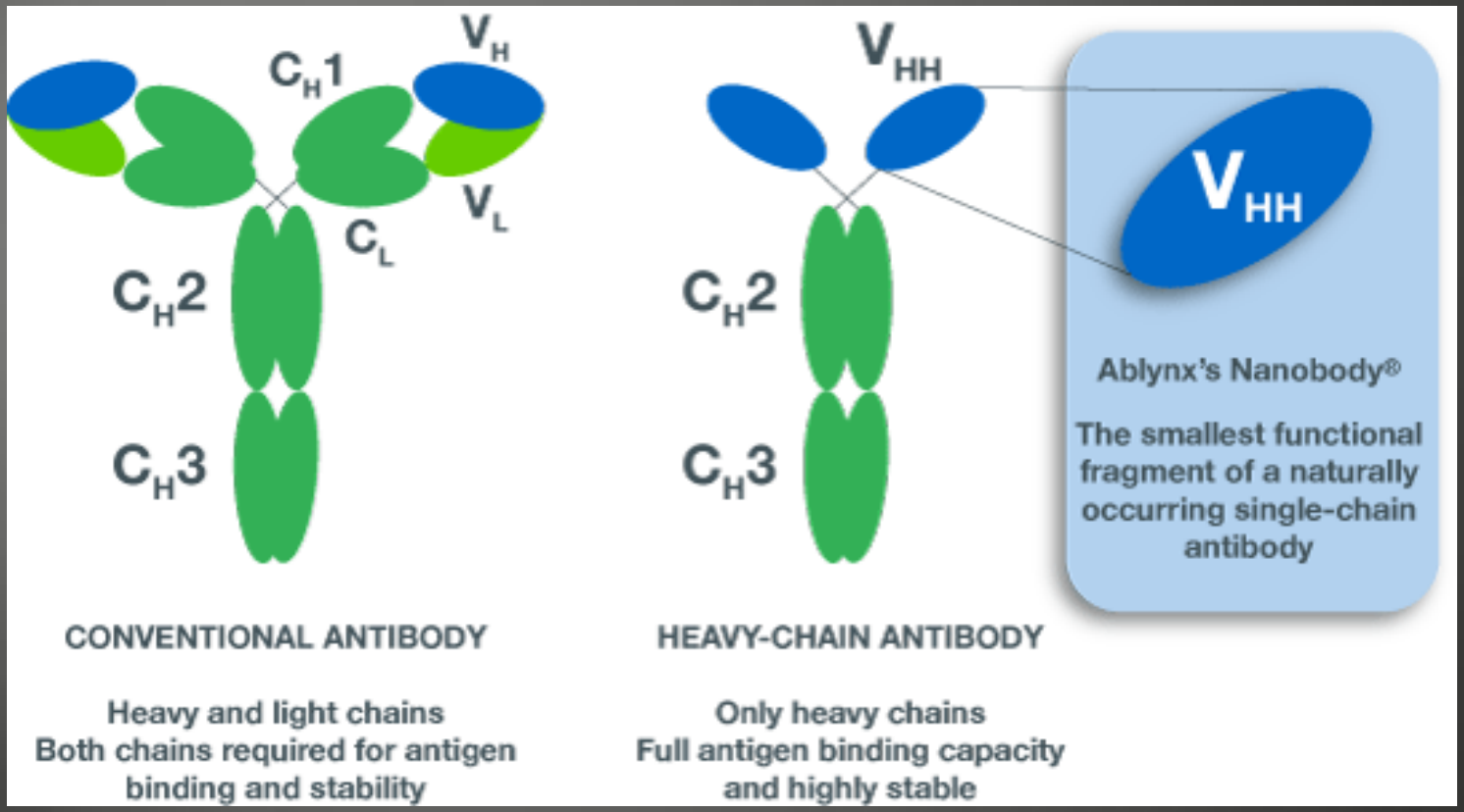
Trastuzumab





Nanobodies

- What are 'nanobodies'?
  - 10 times smaller than conventional antibody



Our research

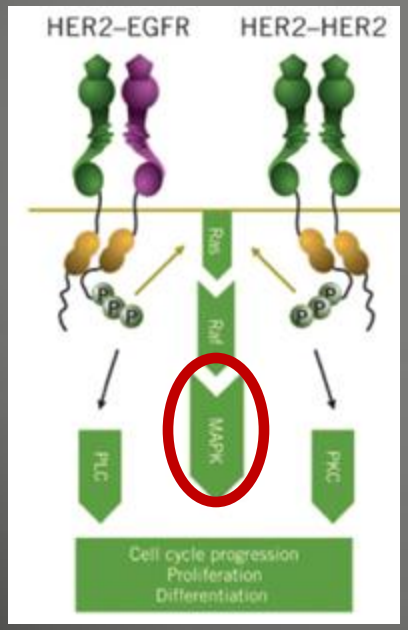
- 3 nanobodies that bind to HER2
- Near-IR fluoresence
- Effect on cancer cells
  - Effect on cell proliferation?
  - Effect on dimer formation?
  - Effect on downstream signalling?
- 3 scenarios:
  - No effect on dimer formation
  - Inhibiting effect on dimer formation
  - Increase in dimer formation

2 cell lines

SKBR3

Overexpressing:

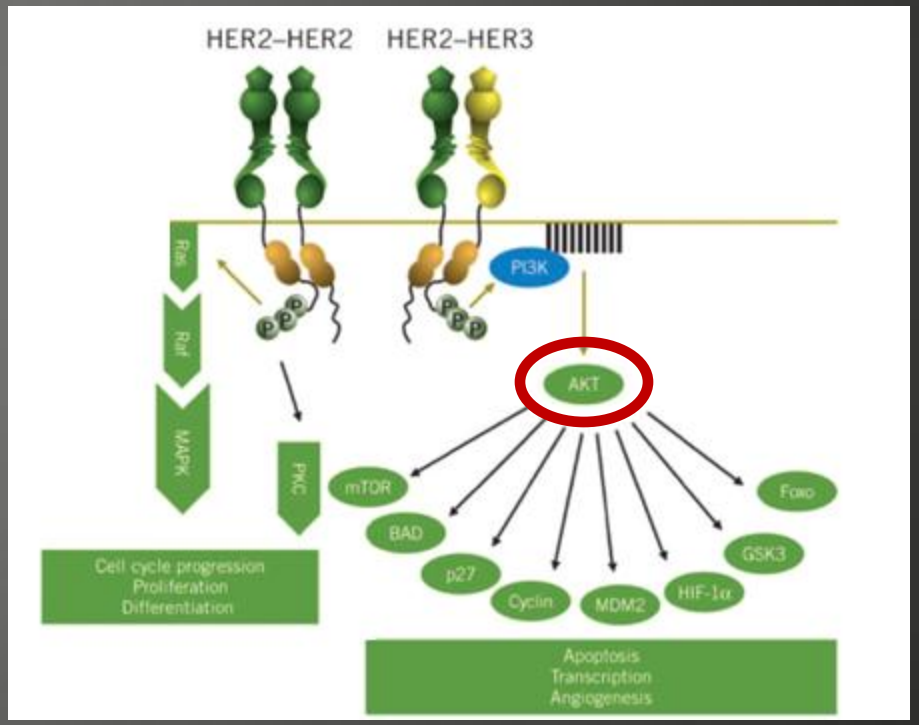
HER-2 & **HER-1** (EGFR)



BT474

Overexpressing:

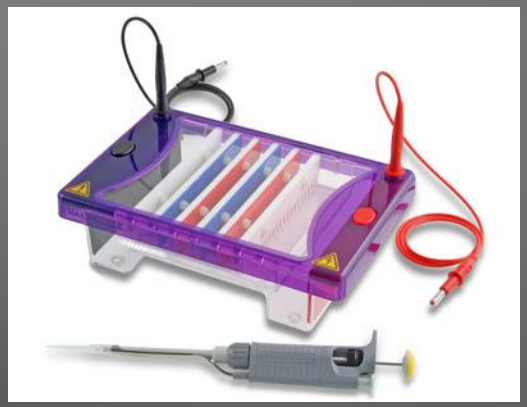
HER-2 & **HER-3**

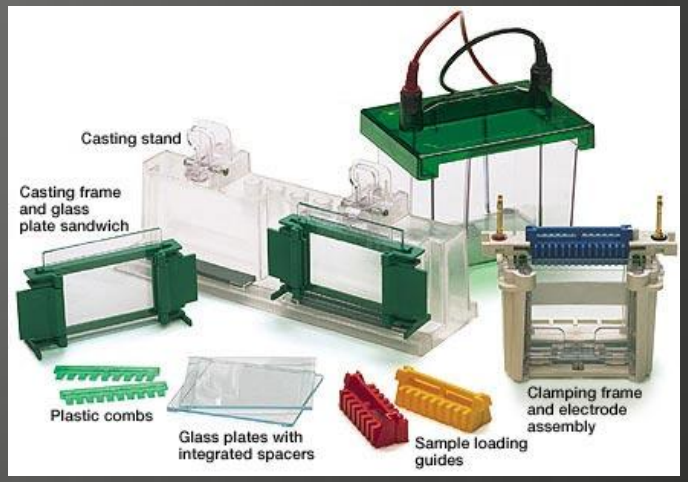


LEE S. ROSEN (2010)

Experimental techniques

- Spectrophotometry
- Gel electrophoresis
- SDS gel electrophoresis
- Immunoprecipitation
- Western blotting





Our research

Effect of VHH on:

- Tumor cell growth?
- Dimer formation?
- Downstream signaling (MAPK/AKT phosphorylation)?

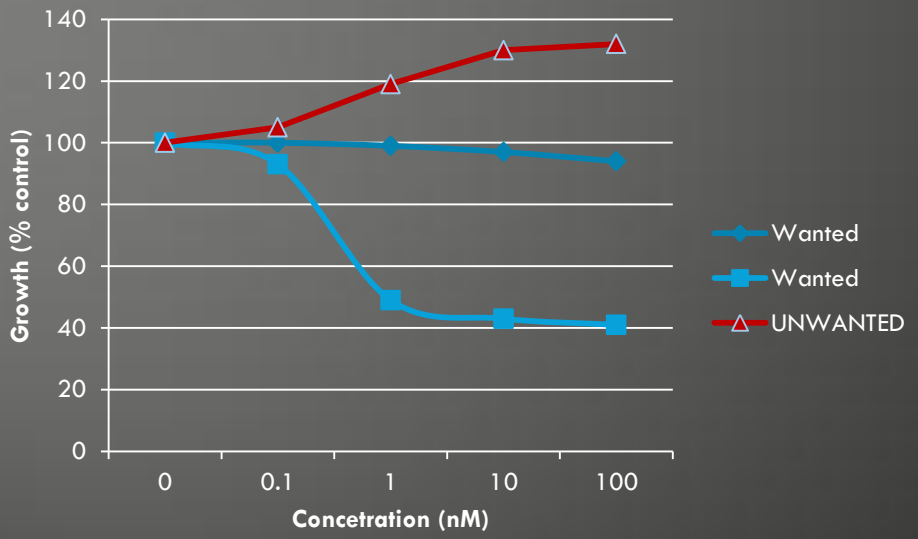
- Extra: Where do nanobodies bind?
  - Competitive binding nanobodies?
  - Trastuzumab?

Experiment 1

- Proliferation assay:
  - 24h starvation of cells
  - Add reagents: Table next slide
  - 48h at 37 degrees
  - Determine relative growth

Experiment 1
Cell Proliferation Assay

	VHH 1	VHH 2	VHH 3	Trastuzumab
1. Control				
2. Control (-)				X
3.	X			
4.		X		
5.			X	



Our research

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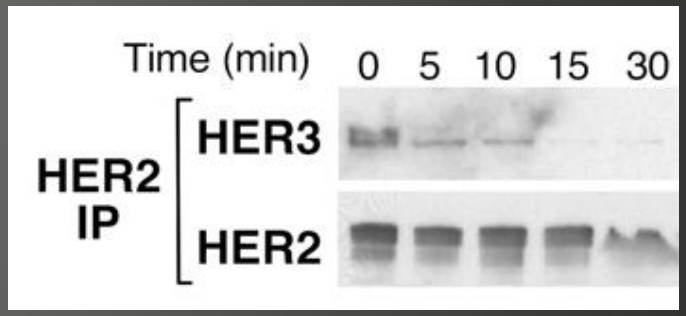
Experiment 2 & 3: treatments

	VHH 1	VHH 2	VHH 3	Ligand (EGF/HRG)	Trastuzumab
1. Control					
2. Control (-)				X	X
3. Control (+)				X	
4.	X				
5.	X			X	
6.		X			
7.		X		X	
8.			X		
9.			X	X	

Experiment 2

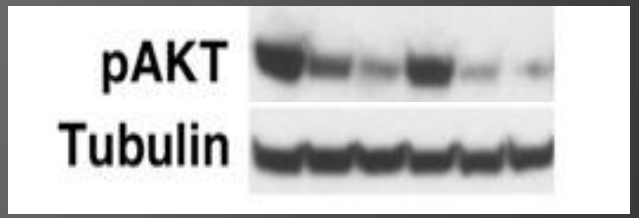
- Dimer formation assay:
  - 24h starvation of cells
  - Incubation with reagents: 15/30/45/60/120 min.
  - Lyse cells
  - Immunoprecipitation
  - Gel electrophoresis, western blotting



(example)

Experiment 3

- Effect on downstream signaling-pathways (MAPK/AKT phosphorylation):
  - 24h starvation of cells
  - Incubation with reagents: 15/30/45/60/120 min.
  - Lyse cells
  - SDS gel electrophoresis, western blotting
  - Loading control: tubulin
  - (p)MAPK and (p)AKT antibodies



(example)

Our research

Effect of VHH on:

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- Extra: Where do nanobodies bind?
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  → ELISA

Relevance

- No. 1 death cause of women between 35-50 years old
- Breast cancer: ~30% Her2 over expression
- Improved imaging and diagnostics
  - Faster, better imaging, monitoring, no need for biopsy, etc.
- Extending pharmacological possibilities
  - Alternative medication
  - Cheaper medication

Sources

- Brockhof, G. *et al.* (2007): **Differential impact of Cetuximab, Pertuzumab and Trastuzumab on BT474 and SK-BR-3 breast cancer cell proliferation,** *Cell Prolif.* 2007 Aug;40(4):488-507
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- Teemu T. Junttila *et al.* (2009): **Ligand-Independent HER2/HER3/PI3K Complex Is Disrupted by Trastuzumab and Is Effectivily Inhibited by the PI3K Inhibitor GDC-0941**, *Cancer Cell*, Volume 15, Issue 5, 429-440, 5 May 2009
- **Centraal bureau voor de statistiek:** cijfers over sterfte borstkanker
- **Genentech, site about biooncology:** http://www.biooncology.com/ general and research part about HER family