UNIKLINIK RWTH AACHEN

Klinik für Nuklearmedizin

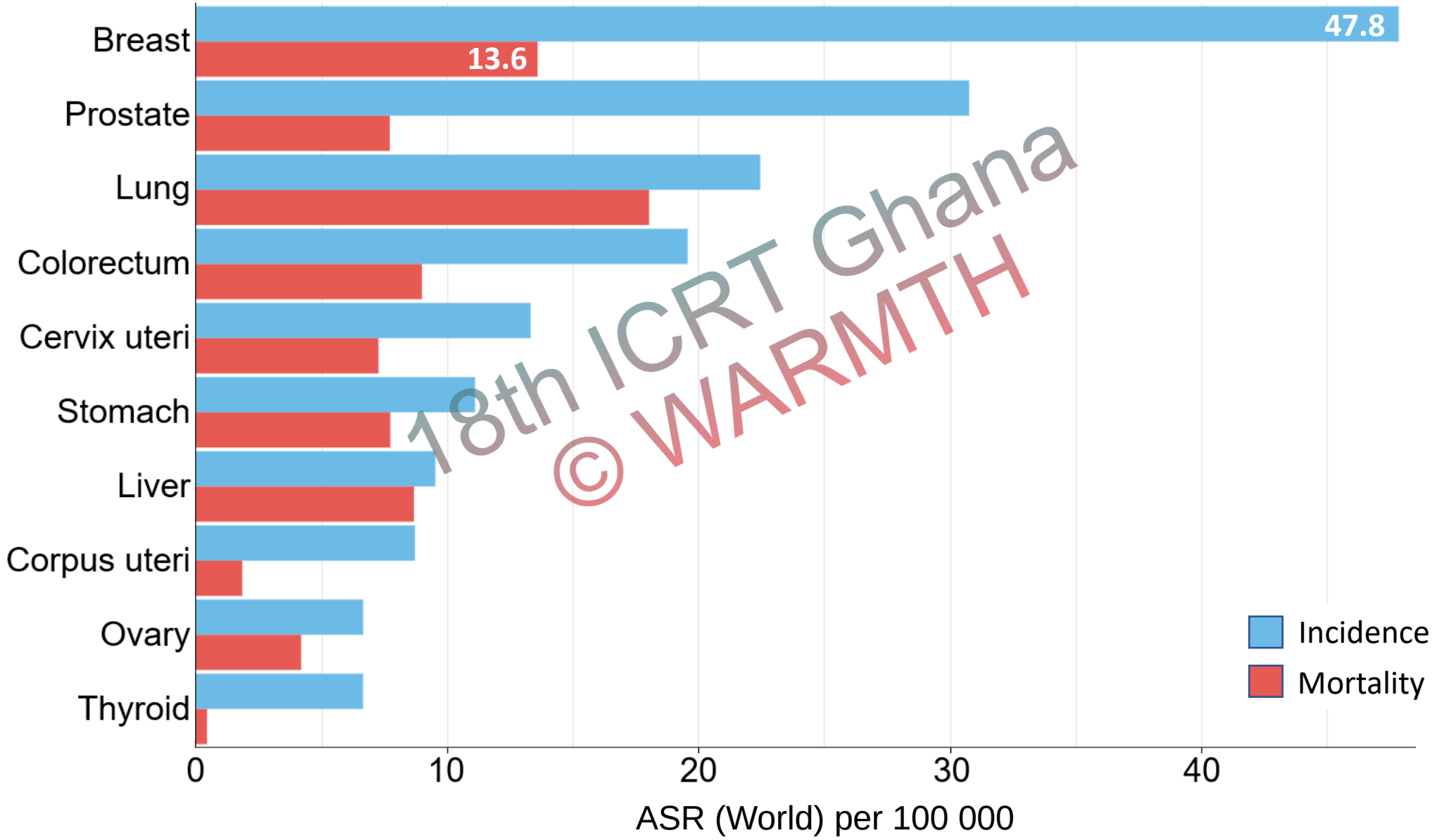
Use of Radionuclide-Based Imaging Methods in Breast Cancer

Betül Altunay

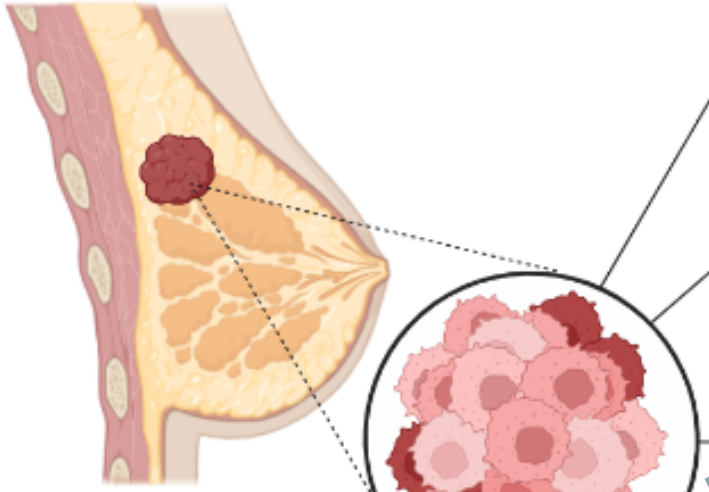
University Hospital RTWH Aachen, Germany

Estimated age-standardized incidence and mortality rates

(World, both sexes, all ages, 2020)



Accurate grouping of Breast Cancer is important



Luminal A (~40%)
HR+ (ER+ and/or PR+), HER2-
- Most prevalent subtype

Normal-like (~2-8%)
HR+ (ER+ and/or PR+), HER2-
- Slightly worse prognosis than Luminal A

Luminal B (~20%)
HR+ (ER+ and/or PR+), HER2+/-
- High levels of Ki-67: fast cancer cell growth

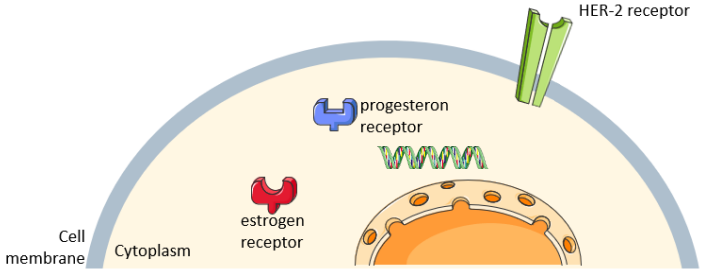
HER2-enriched (~10-15%)
HR- (ER-, PR-), HER2+
- Faster growth than luminal subtypes

Triple Negative (~15-20%)
HR- (ER-, PR-), HER2-
- Most aggressive subtype
- Occurs more often in younger women
- Highest association to BRCA1 mutations

Best prognosis

Worst prognosis

Receptors:



3Biorender.com
Johnson et al. (2021) JBI

Receptor testing strategies

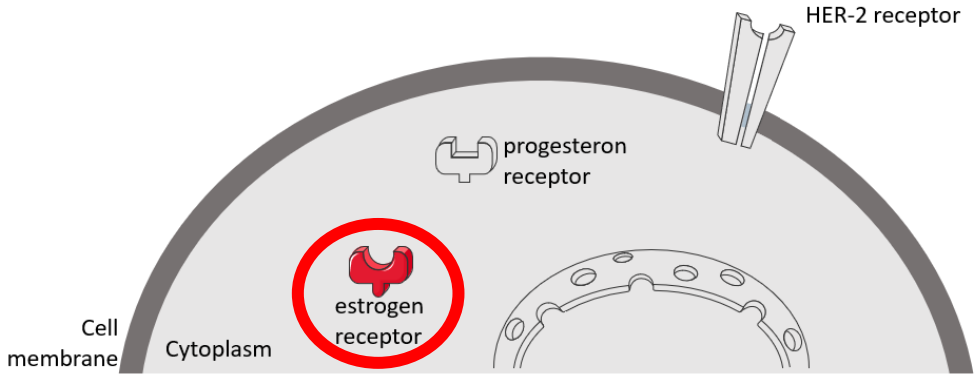
- Standard: IHC (protein) and FISH (gene)
- Protein amount: ELISA, Western Blot
- Gene amplification: CISH, SISH, Southern blot, and PCR

– need for biopsy
  - Not always attainable or desired
  - Multiple samples necessary to detect inter- and intratumoral heterogeneity as well as treatment-related changes in receptor expression over time
  - IHC results alone are not reliable, inaccurate results

- FDG PET scan
  - only metabolism

Altunay et al. (2020) EJNMMI

Imaging Probes Targeting Estrogen Receptor

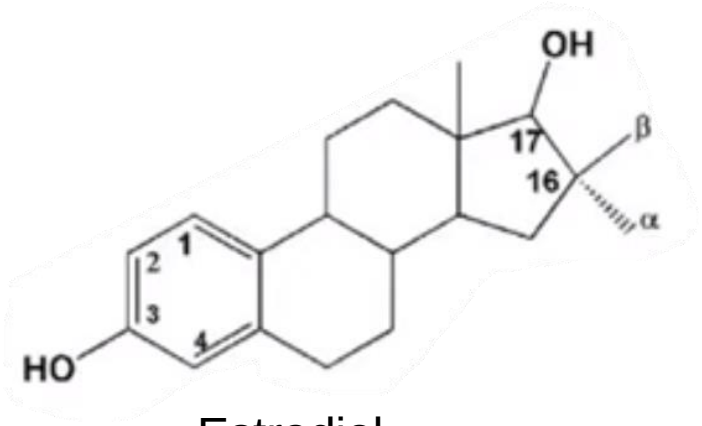


Estrogen receptor (ER)

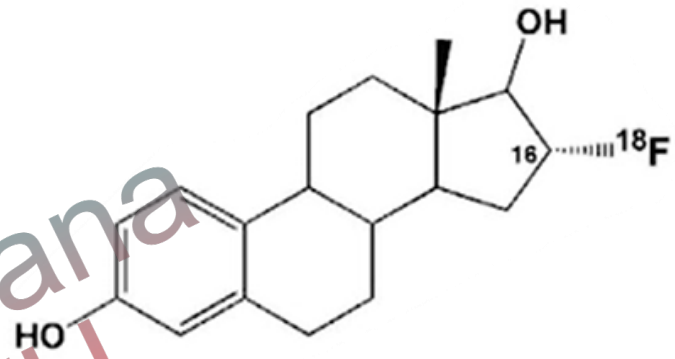
- A member of the steroid receptor family
- Expressed at high levels in 70-80% of BCs
- **ERα** (1960) & ERβ (1996) are activated by the binding of estradiol
- metastasis formation is promoted by deregulation of ER co-regulators or extra-nuclear ERα signaling

Rozeboom et al. (2019) Am J Cancer Res; Jensen et al. (1962) Perspect Biol Med; Kuiper et al. (1996) Proc Natl Acad Sci

16α-[^{18}F] fluoroestradiol (FES)*



Estradiol



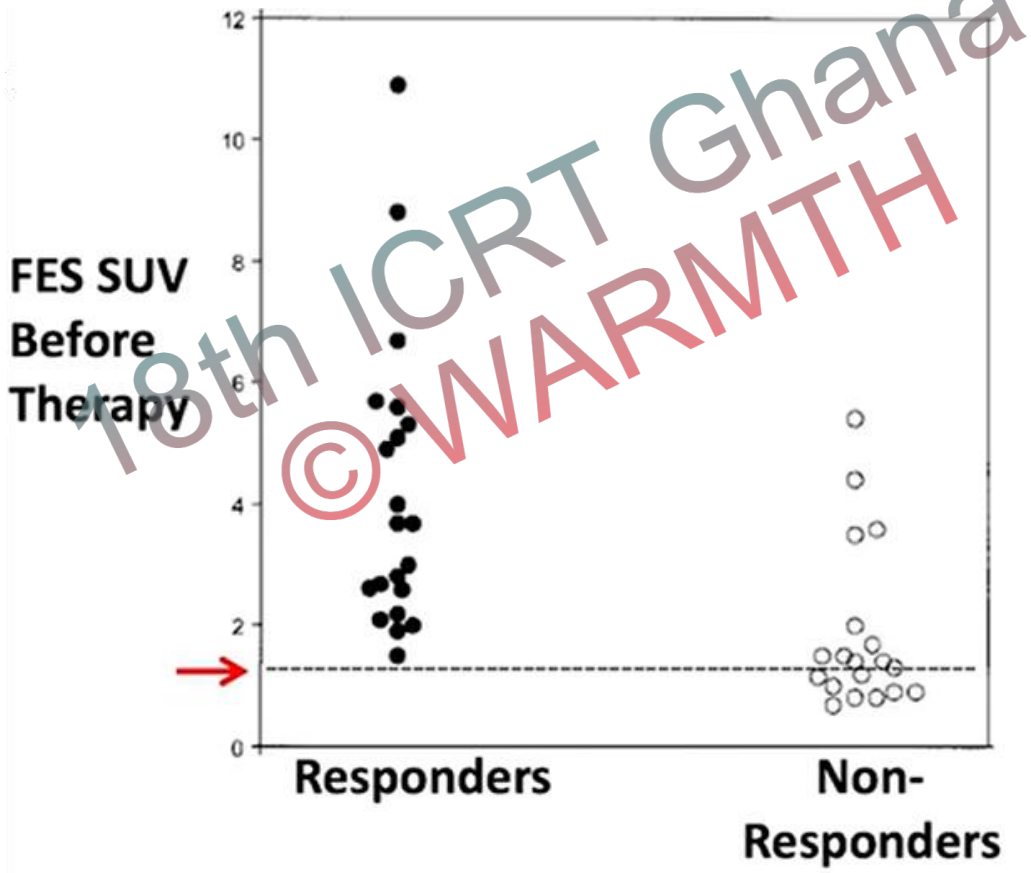
16α-[^{18}F] fluoroestradiol

- first PET imaging agent for a receptor target in cancer (1984)
- High correlation with the ER expression determined by IHC
- Approved by the U.S. FDA since May 2020 (Cerianna™, sold by GE Healthcare)

Kiesewetter et al (1984) J Nucl Med

16α-[^{18}F] fluoroestradiol (FES)*

1) Determine ER status in metastatic lesions
→ Should ER therapies be used?



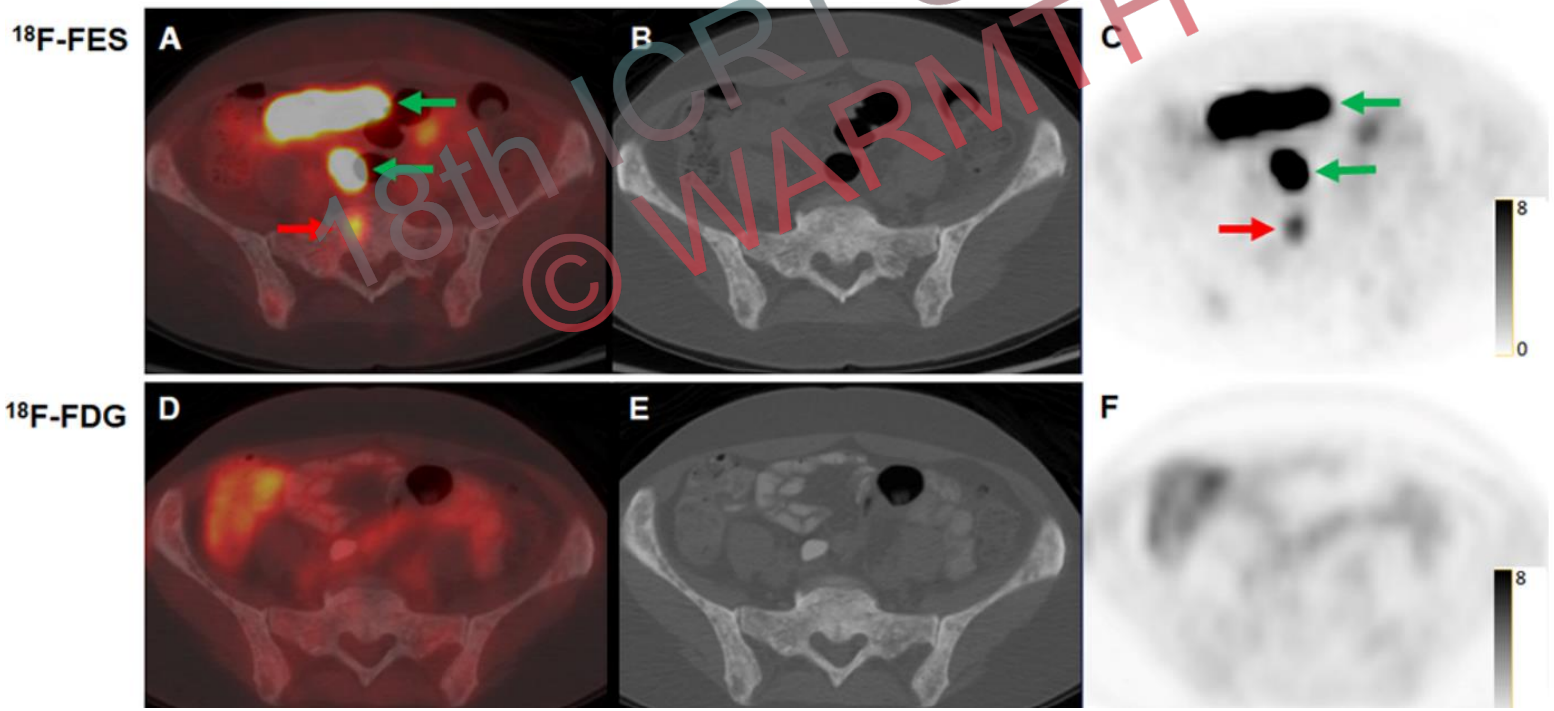
Study design
40 women
Biopsy-proven ER+ BC
7-10 days tamoxifen therapy

Mortimer et al. (2001) J Clin Oncol

16α-[^{18}F] fluoroestradiol (FES)*

1) Determine ER status in metastatic lesions
   → Should ER therapies be used?

2) Invasive lobular breast cancer
   → systemic staging, detection of disease recurrence

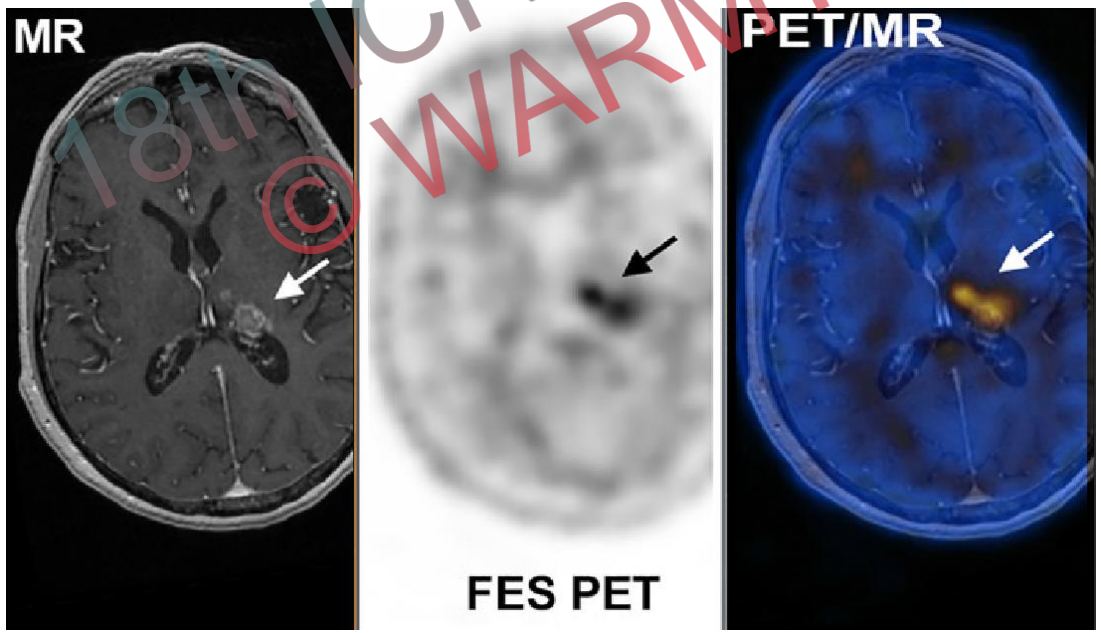


<u>Study design</u>
Retrospective analysis
Biopsy-proven ILC

Ulaner et al. (2020) JNM

16α-[^{18}F] fluoroestradiol (FES)*

1) Determine ER status in metastatic lesions
   → Should ER therapies be used?
2) Invasive lobular breast cancer
   → systemic staging, detection of disease recurrence
3) Problem solving tool
   → Ambiguous findings on other imaging procedures



<u>Study design</u>
Meta analysis
ER+ MBC

Kurland et al. (2020) The Oncologist

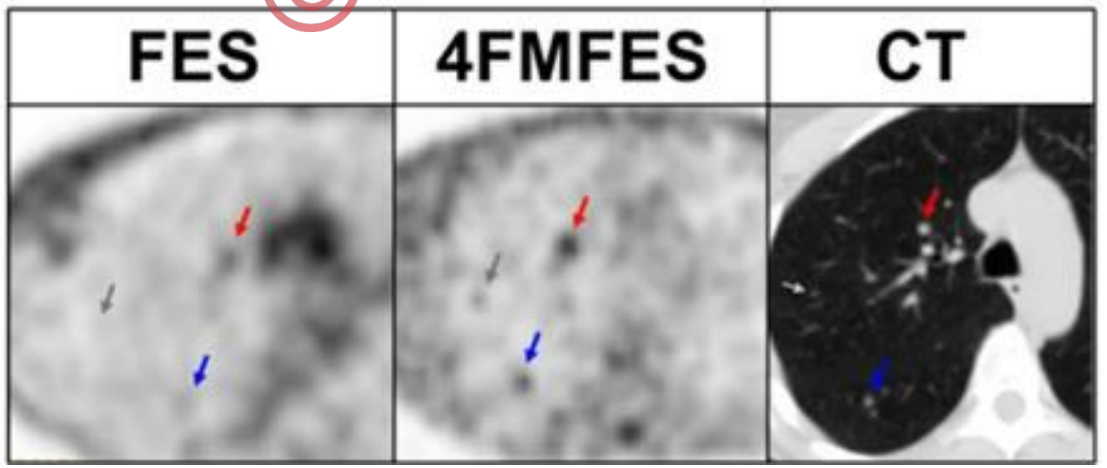
16α-[^{18}F] fluoroestradiol (FES)*

1) Determine ER status in metastatic lesions

- slow blood clearance and rapid metabolism in the liver
- Hepatobilliary and urinary excretion
- Affinity to plasma globulins

→ Ambiguous findings on other imaging procedures

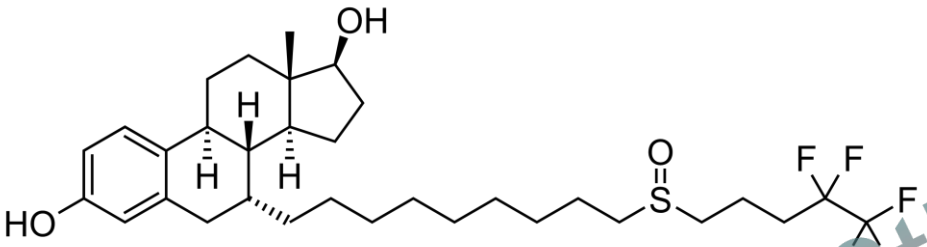
4-fluoro-11βmethoxy-16α-[^{18}F]fluoroestradiol (^{18}F-4FMFES)



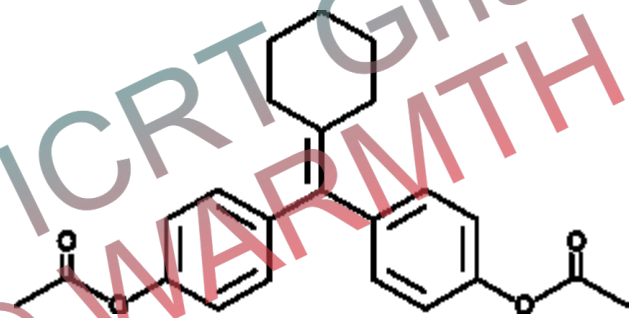
Study design
n = 31
ER+ BC

Paquette et al. (2018) JNM

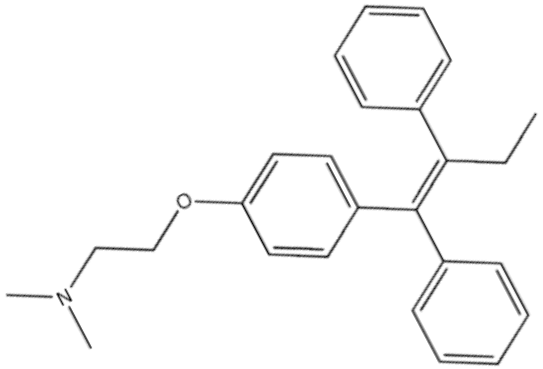
Endocrine-Drug Based ER Imaging Probes



Fulvestrant



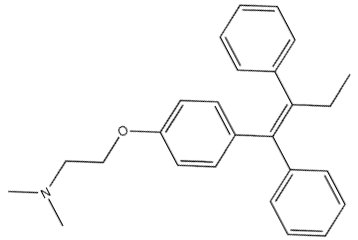
Cyclofenil



Tamoxifen

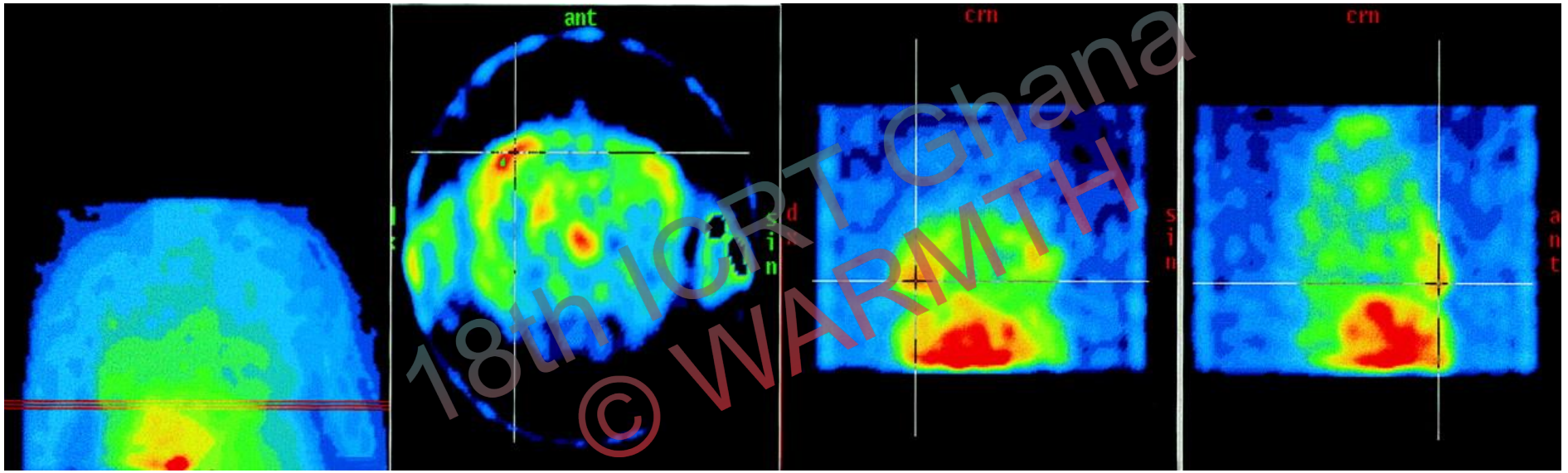
Seimbille et al. (2004) Nucl Med Biol; Seo et al. (2007) Nucl Med Biol

[^{123}I]iodotamoxifen (^{123}I-TX)



Tamoxifen

MIP transaxial coronal sagittal

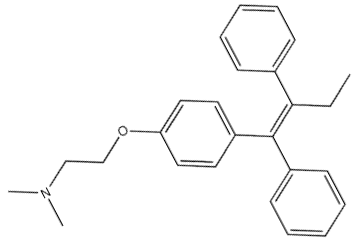


<u>Study design</u>:
30 min p.i.
n = 9
4/6 ER+/PR+
0/2 ER+/PR-
0/1 ER-/PR-

- uptake in primary breast tumor
- Physiologic uptake in homolateral and contralateral noninvolved breast tissue, as well as persisting high (intracardiac) blood-pool activity

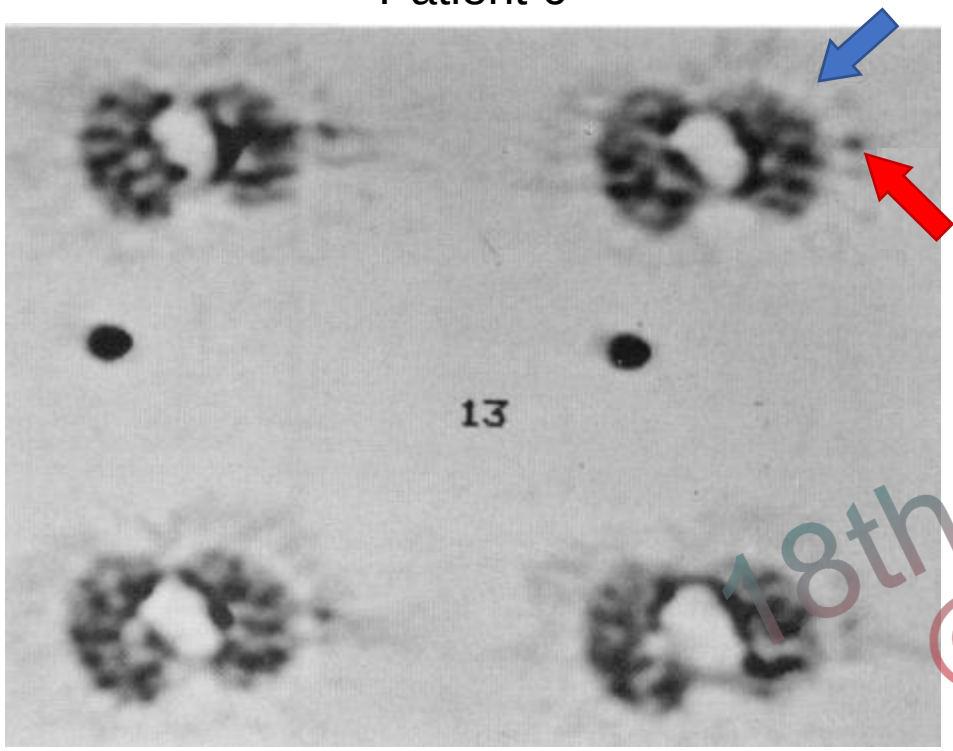
Van de Wiele et al. (2001) JNM

[^{18}F]fluorotamoxifen ([^{18}F]FTX)



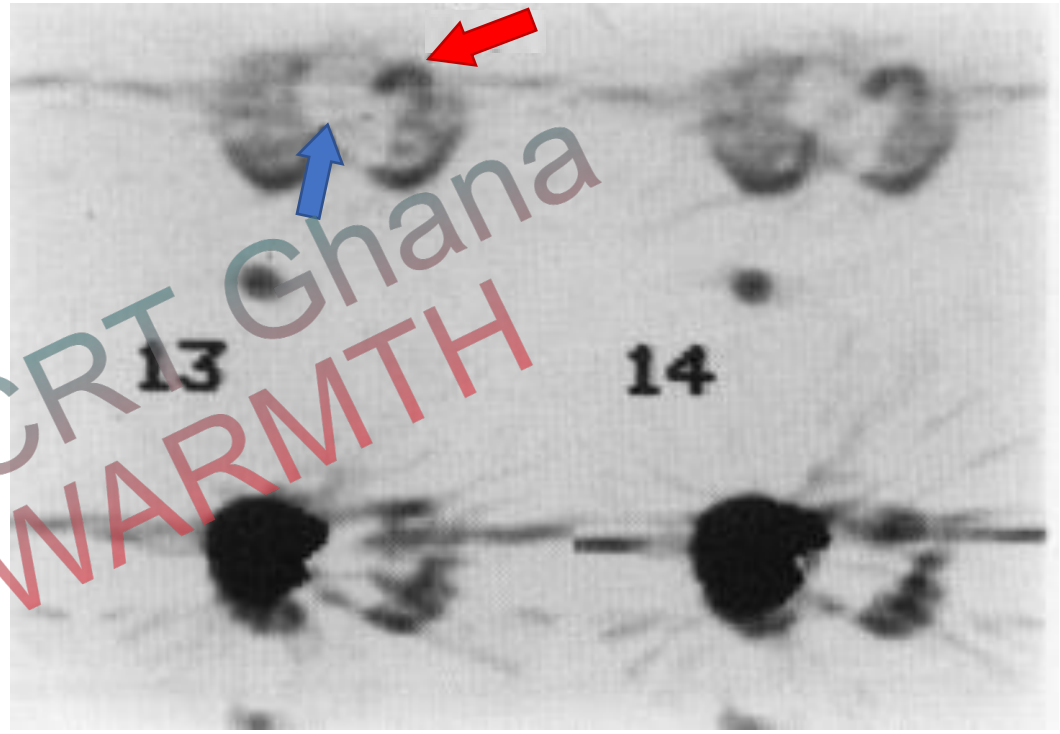
Tamoxifen

Patient 6



- Focal increased uptake in the left axilla
- no focal abnormality in the left breast

Patient 2



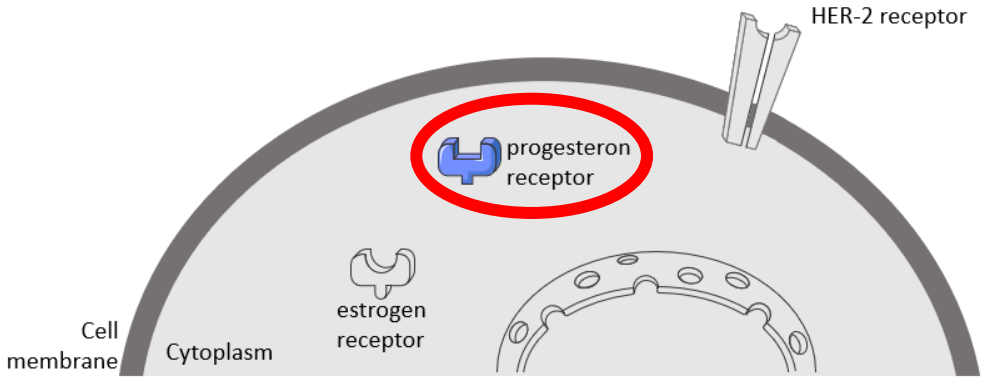
- High uptake in the lung, heart and liver
- no significant uptake in the metastatic lesion

→ poor response to tamoxifen therapy

<u>Study dessign:</u>
60-80 min p.i.
n = 10

Inoue et al. (2009) JBR

Imaging Probes Targeting Progesterone Receptor



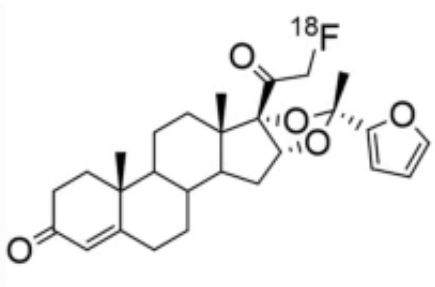
Progesterone receptor (PR)

- PR+/ER+ is seen in 65 - 75% of BCs
- ER-/PR+ is seen in less than 2% of BCs
- Two predominant PR isoforms: PR-A and PR-B
- PR expression is regulated by the ER and its activity by an estrogen-related gene
- the knowledge about PR is still incomplete → only limited progress in PR imaging

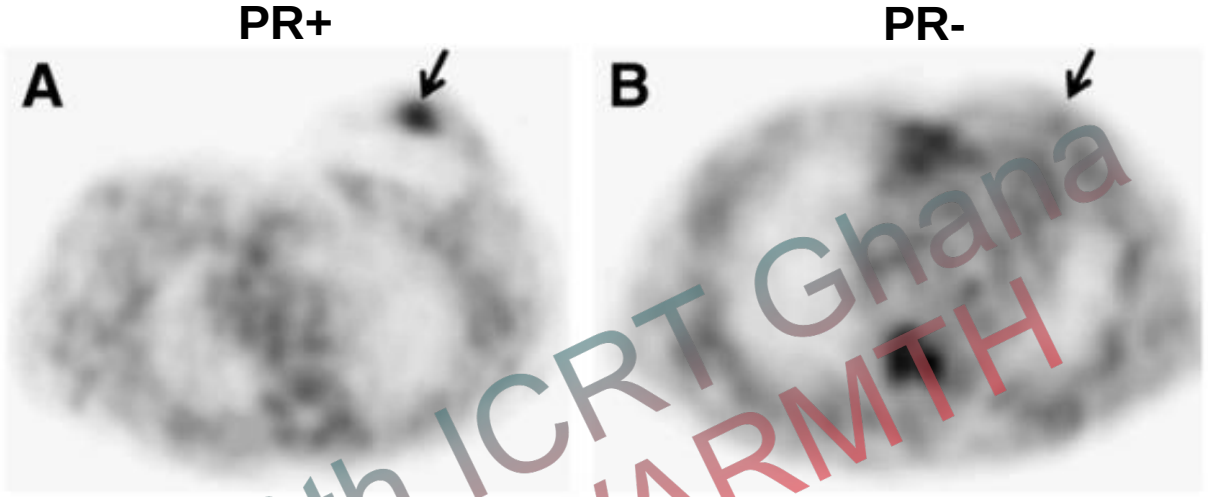
Li et al. (2020) Oncology; Trabert et al. (2019) Endocr. Rev.

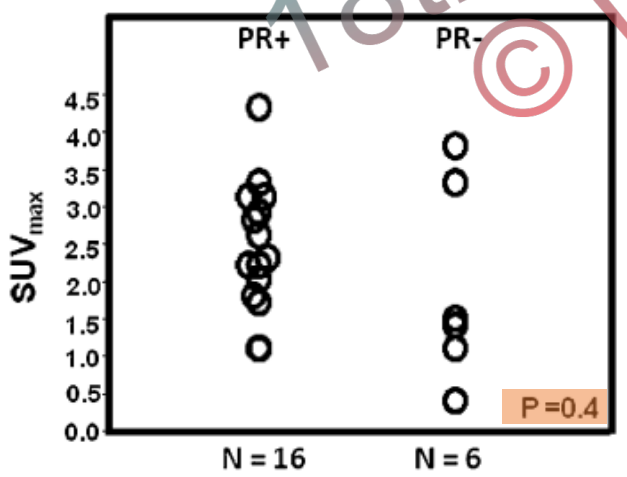
[^{18}F]FFNP

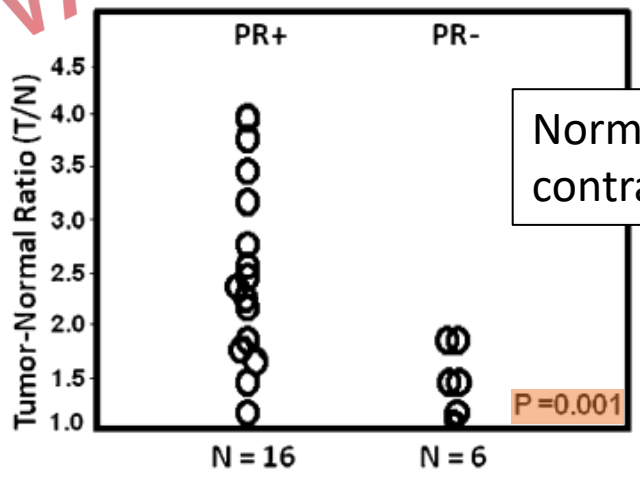
21-^{18}F-fluoro-16α, 17 α -[(R)- (1'-α-furylmethylidene)-19-norpregn-4-ene-3, 20-dione



[^{18}F]FFNP





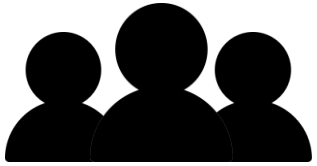


→ Accurate determination of PR status

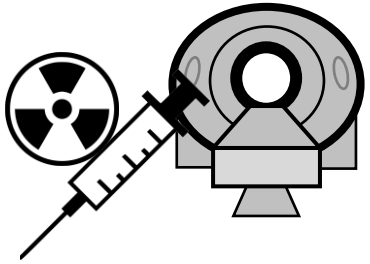
Normal = contralateral breast

Dehdashti et al. (2011) JNM

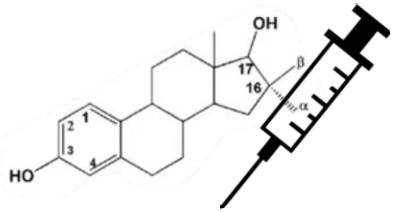
[^{18}F]FFNP (clinical phase II)



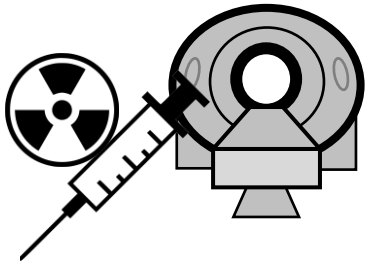
43 postmenopausal women with advanced ER-positive breast cancer



approx. 340 MBq 18F-FFNP; PET/CT



three doses of estrogen over a 24-hour period (total dosage of 6 mg)



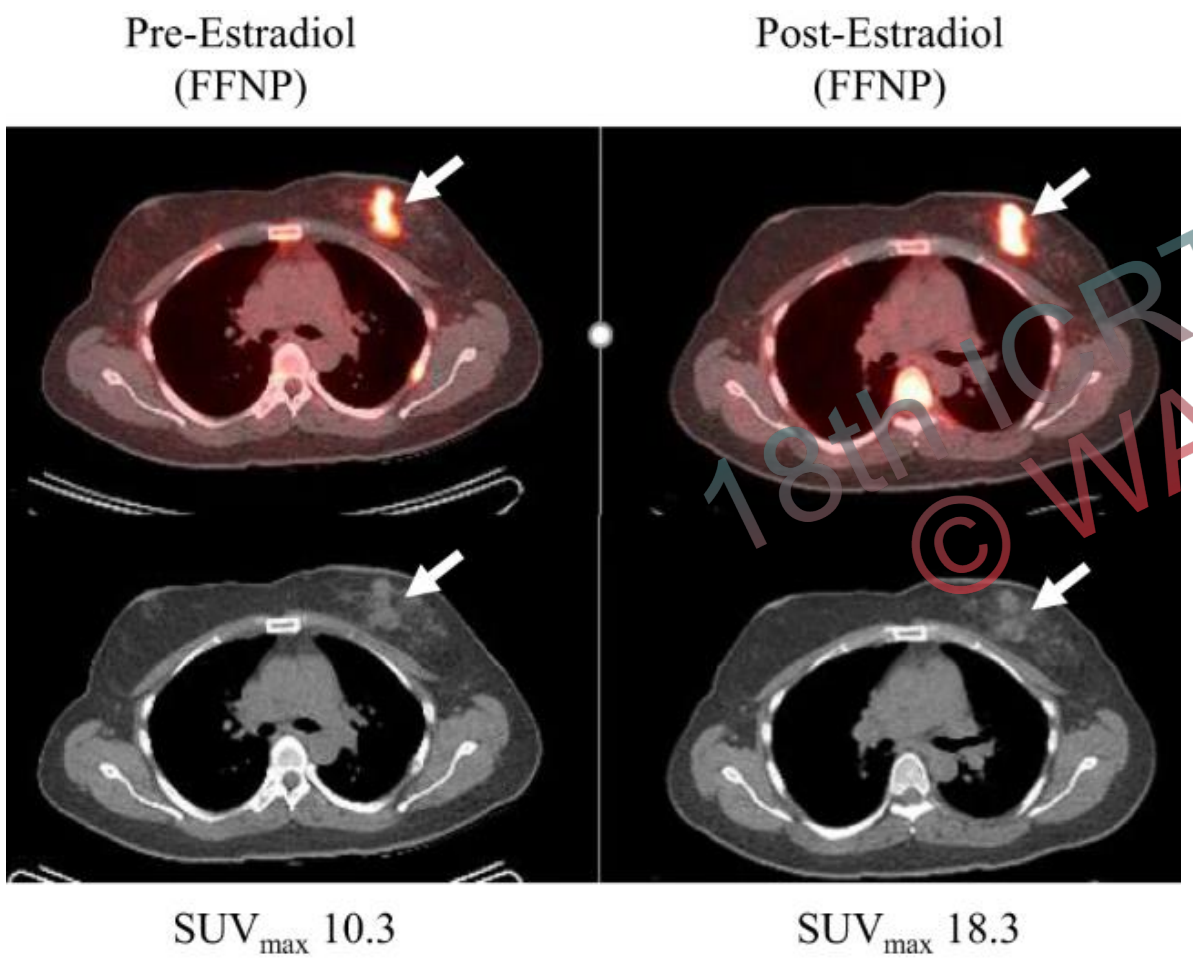
approx. 340 MBq 18F-FFNP; PET/CT

Endocrine therapy as recommended by their oncologist

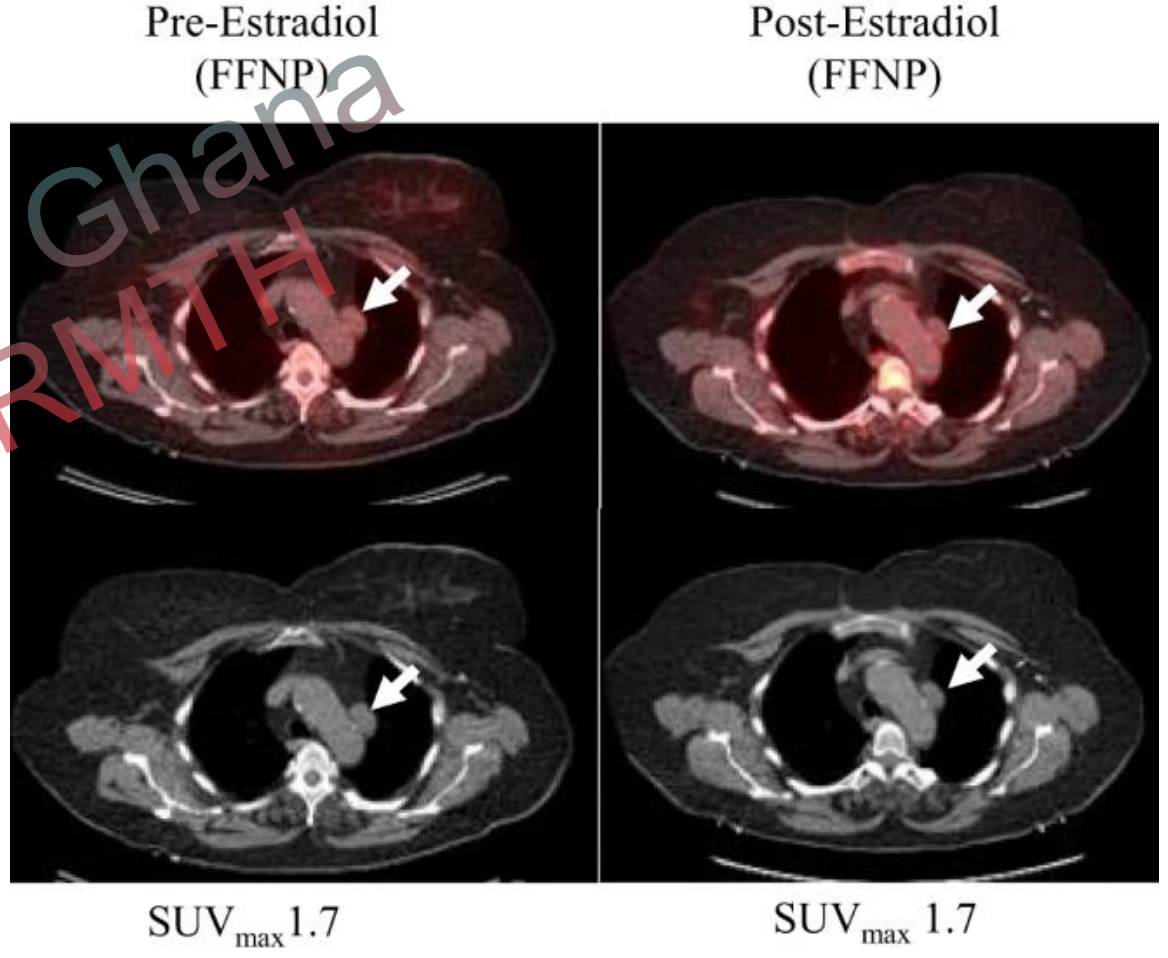
Dehdashti et al. (2021) Nat. Commun

[^{18}F]FFNP

Responder (n=28)

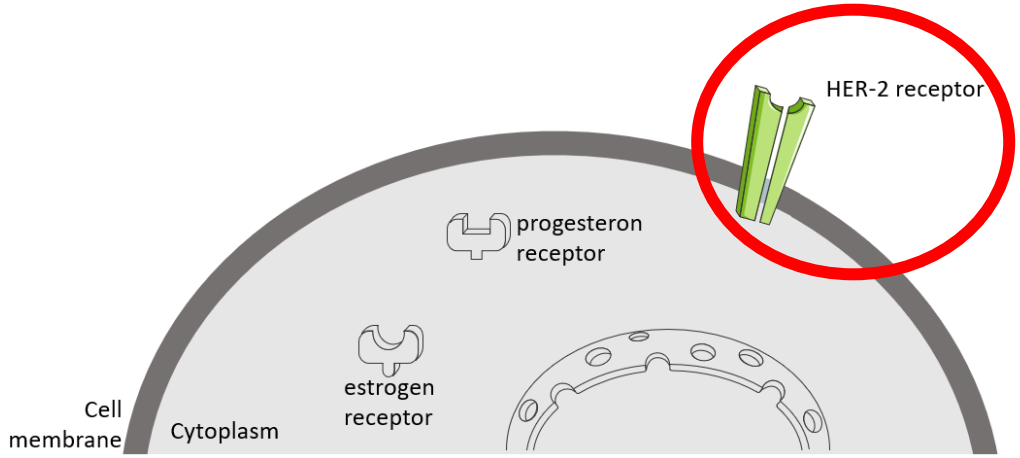


Nonresponder (n=15)



Dehdashti et al. (2021) Nat. Commun

Imaging Probes Targeting HER2

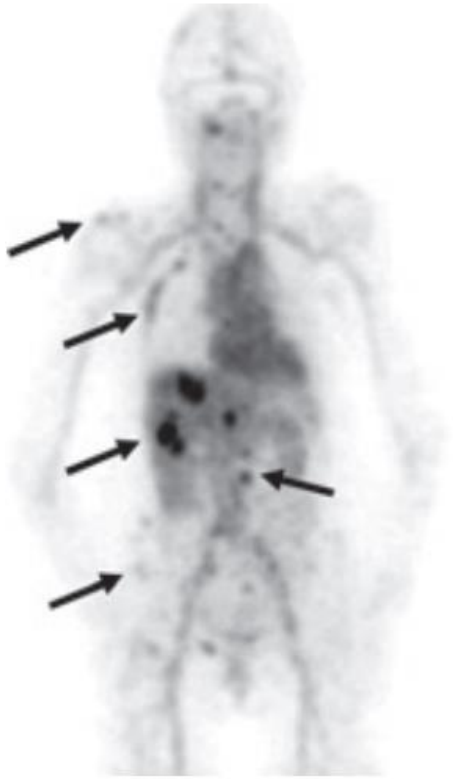


Human Epidermal Growth Factor Receptor 2 (HER2)

- 18 - 20% of BC
- 2 millions time higher HER2 protein expression
- Activation triggers cell growth, differentiation, survival and migration

Altunay et al. (2020) EJNMMI

Radiolabelled mAb: ^{89}Zr-Trastuzumab

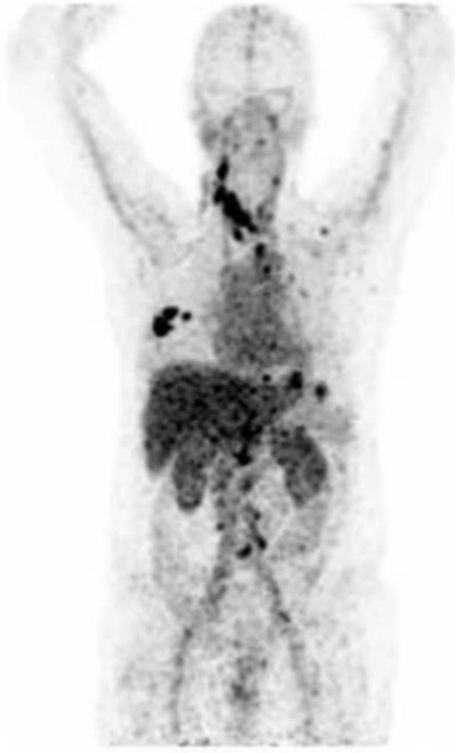


- Imaging 5 days p.i.
- 6/12 received sufficient dose
- low sensitivity and no biopsies to confirm specificity

Dijkers et al. (2010) Clin Pharmacol Ther.

- detects HER2+ metastasis in IHC confirmed HER2- primary BC

Ulaner, 2017, Clin Nucl Med



- Imaging 4 days p.i.
- Low sensitivity
- Liver and spleen had higher uptake

Bensch et al. (2018) EJNMMI

Disadvantage of radiolabelled mABs

- relatively high molecular weight (~ 160 kDa) of antibodies → cannot be filtered by the kidney and accumulate in the liver, leading to hepatotoxicity
- the heterogeneous blood perfusion
- the hindered diffusion in the interstitium
- the extravascular binding of monoclonal antibodies
- the increased interstitial pressure (turgor effect) leads to a heterogeneous distribution of the antibodies in the tumor
- the high affinity of the antibodies impedes homogeneous tumor penetration and intratumoral diffusion, as the agent can get stuck at the periphery
- This incomplete tumor penetration leads to a suboptimal therapeutic efficiency→ development of resistance
- slow blood clearance (between few days and weeks) → good contrast images only after hours or days after application (long-lived radionuclides)
- considerable degree of non-specific uptake at the target sites, especially at the earlier time-points
- can only be administered intravenously or subcutaneously due to their low thermodynamic stability
- phagocytosis is mediated by the Fc region of the mABs → reducing the availability of the antibody on the cell surface to unfold its desired mechanism of action
- only few antibodies are able to cross the blood-brain barrier and reach the central nervous system

Altunay et al. (2020) EJNMMI

Disadvantage of radiolabelled mABs

- relatively high molecular weight (~ 160 kDa) of antibodies → cannot be filtered by the kidney and accumulate in the liver, leading to hepatotoxicity
- the heterogeneous blood perfusion
- the hindered diffusion in the interstitium
- the extravascular binding of monoclonal antibodies
- the increased interstitial pressure (turgor effect) leads to a heterogeneous distribution of the antibodies in the tumor
- **the high affinity of the antibodies impedes homogeneous tumor penetration and intratumoral diffusion, as the agent can get stuck at the periphery**
- resistance
- slow blood clearance (between few days and weeks) → good contrast images only after hours or days after application (long-lived radionuclides)
- considerable degree of non-specific uptake at the target sites, especially at the earlier time-points
- can only be administered intravenously or subcutaneously due to their low thermodynamic stability
- phagocytosis is mediated by the Fc region of the mABs → reducing the availability of the antibody on the cell surface to unfold its desired mechanism of action
- only few antibodies are able to cross the blood-brain barrier and reach the central nervous system

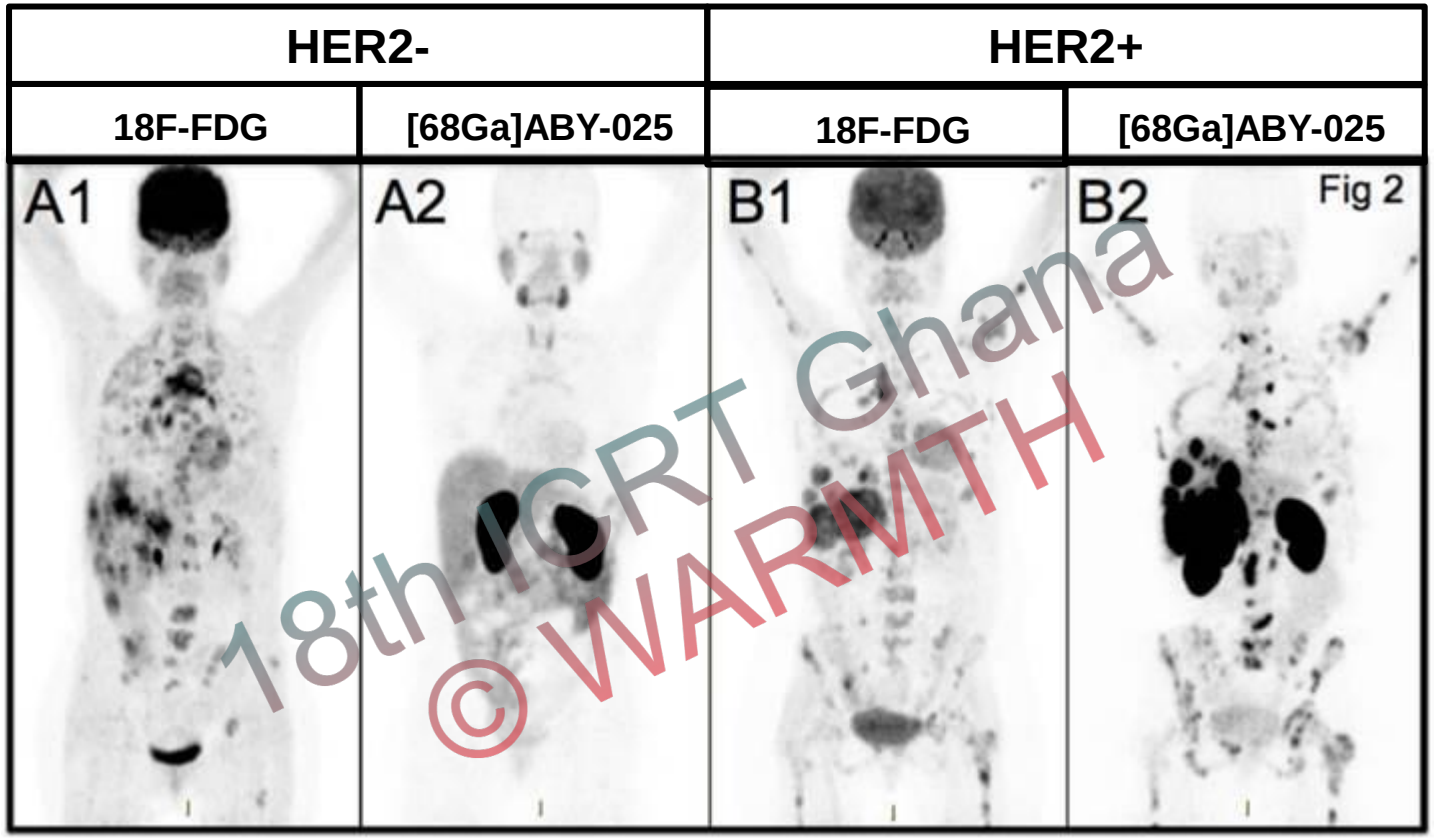
Altunay et al. (2020) EJNMMI

Disadvantage of radiolabelled mABs

- relatively high molecular weight (~ 160 kDa) of antibodies → cannot be filtered by the kidney and accumulate in the liver, leading to hepatotoxicity
- the heterogeneous blood perfusion
- the hindered diffusion in the interstitium
- the extravascular binding of monoclonal antibodies
- the increased interstitial pressure (turgor effect) leads to a heterogeneous distribution of the antibodies in the tumor
- the high affinity of the antibodies impedes homogeneous tumor penetration and intratumoral diffusion, as the agent can get stuck at the periphery
- This incomplete tumor penetration leads to a suboptimal therapeutic efficiency→ development of resistance
- slow blood clearance (between few days and weeks) → good contrast images only after hours or days
- can only be administered intravenously or subcutaneously due to their low thermodynamic stability
- phagocytosis is mediated by the Fc region of the mABs → reducing the availability of the antibody on the cell surface to unfold its desired mechanism of action
- only few antibodies are able to cross the blood-brain barrier and reach the central nervous system

Altunay et al. (2020) EJNMMI

Radiolabelled Affibodies: ^{68}Ga-ABY-025

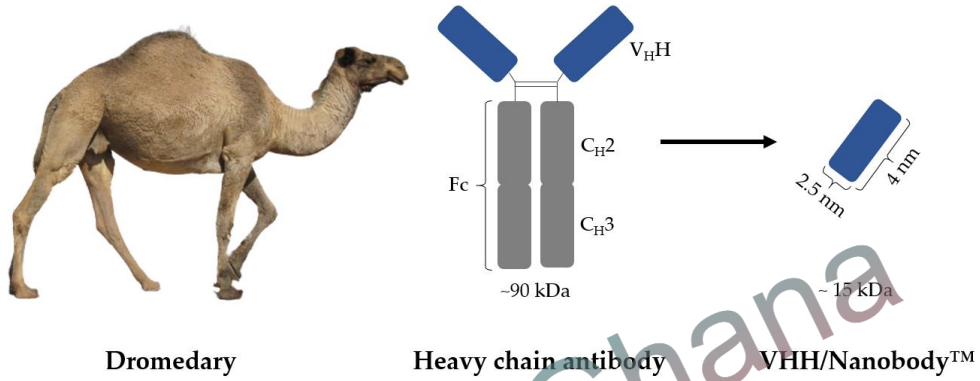


Study design
Clinical phase 1 +2
n = 16
11 HER2+
5 HER2-

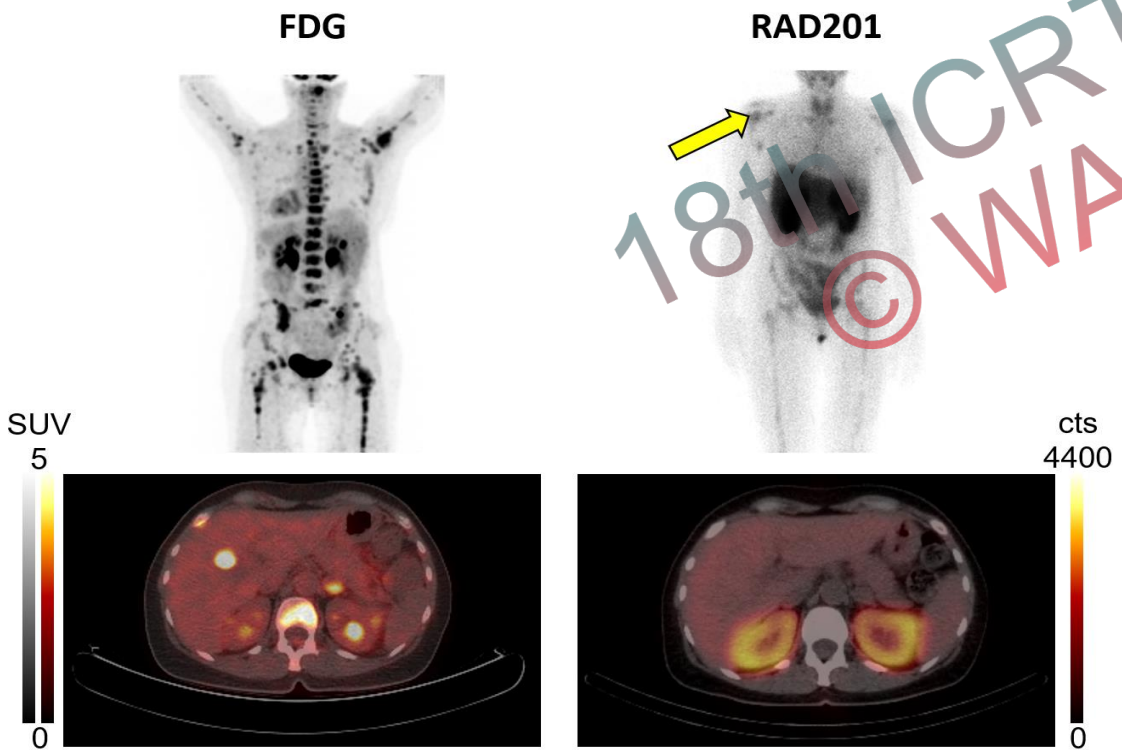
- Imaging 4h after injection
- uptake was five times higher in HER2+ than in HER2- lesions
- allowed differentiation between metastases with the HER2 expression levels of score 3+ and score 2+

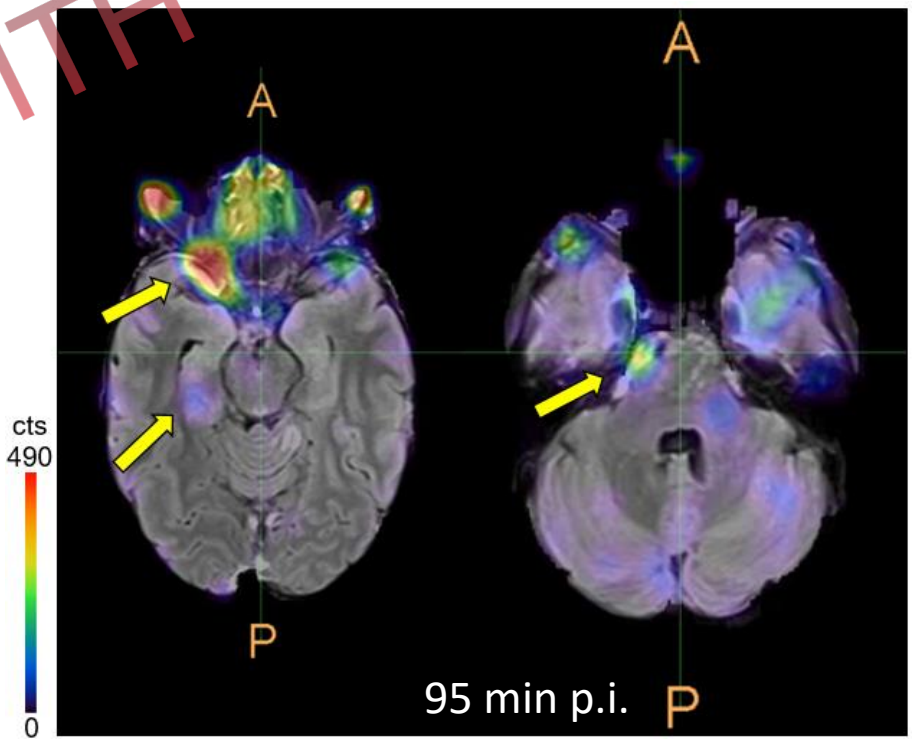
Sörensen et al. (2016) Theranostics

Radiolabelled Nanobodies: RAD201



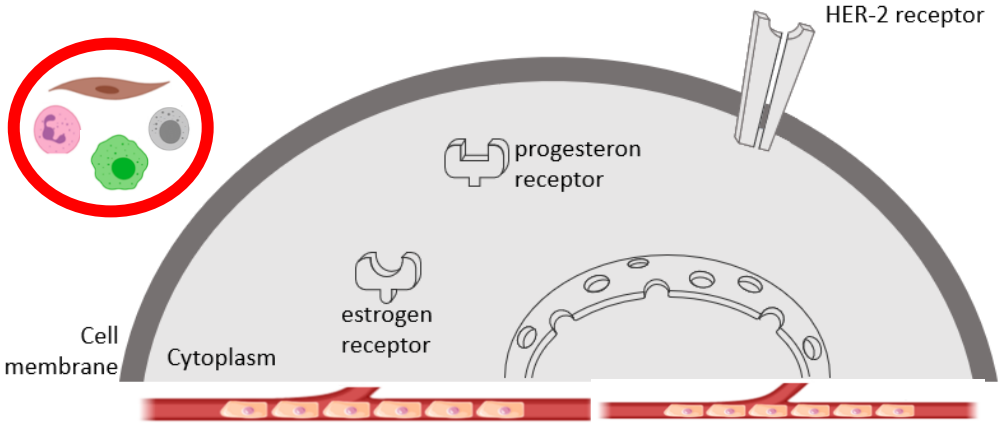
Dromedary | Heavy chain antibody | VHH/Nanobody™





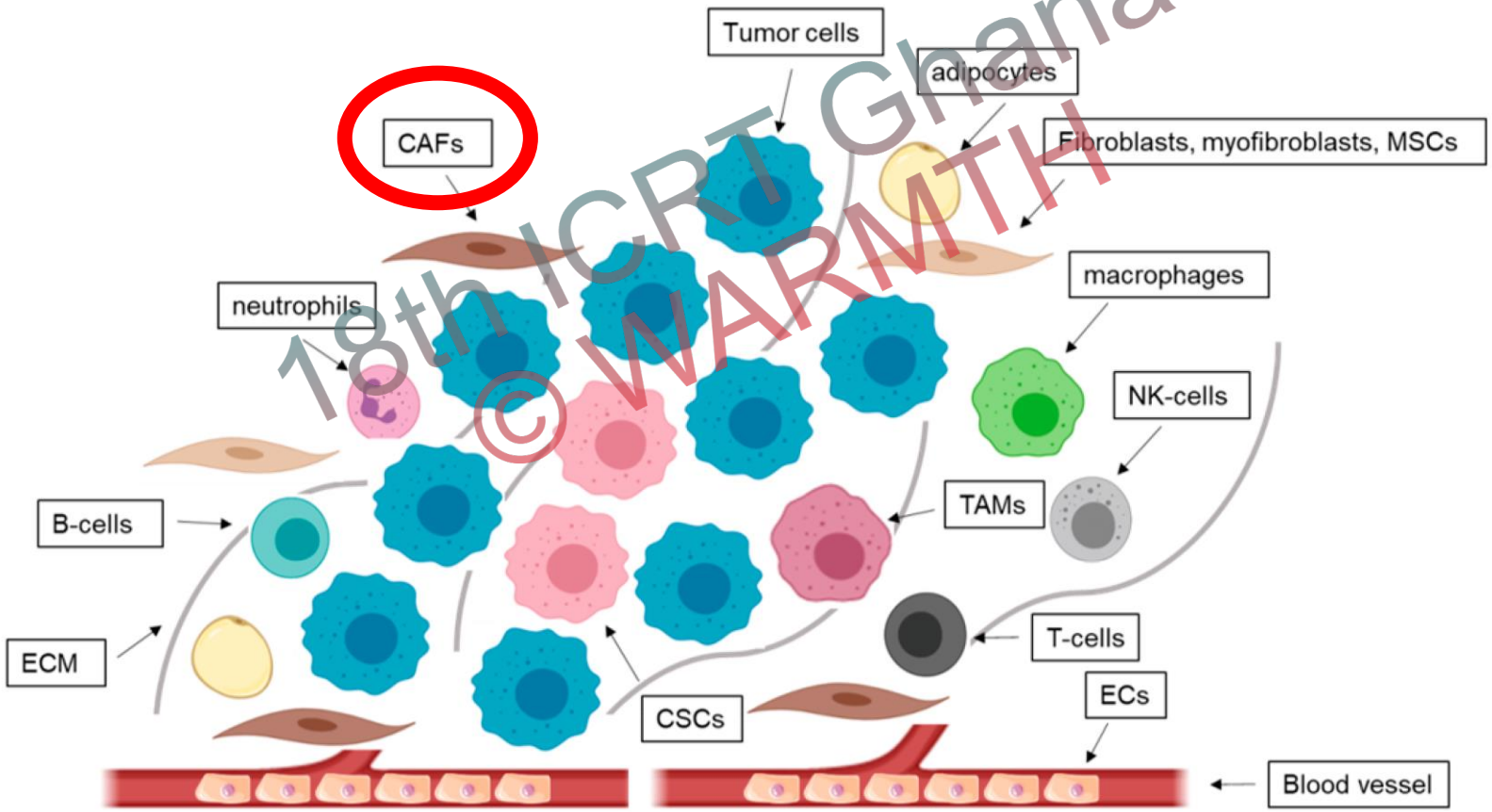
Altunay et al. (2020& 2022) EJNMMI

Imaging Probes Targeting the Tumor Microenvironment



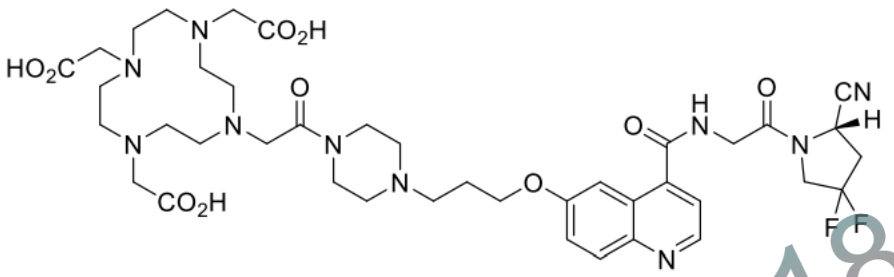
Tumor Microenvironment

- cancer-associated fibroblasts make up to 70% of the whole breast tumor volume
  - fibroblast activation protein (FAP): cell-surface serine protease and highly upregulated in CAFs

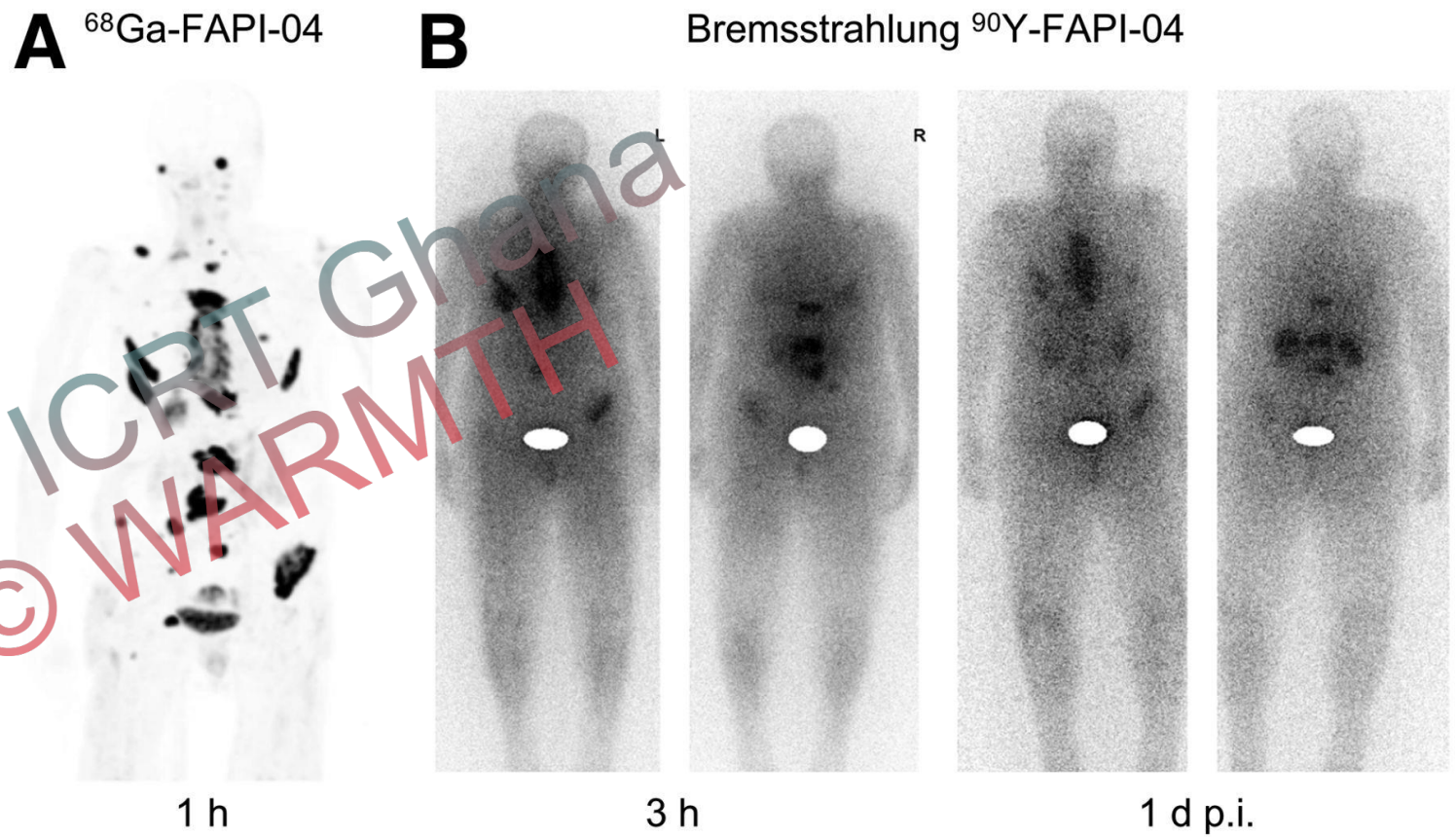


Heesch et al. (2020) Cells

^{68}Ga-/^{90}Y-FAPI-04



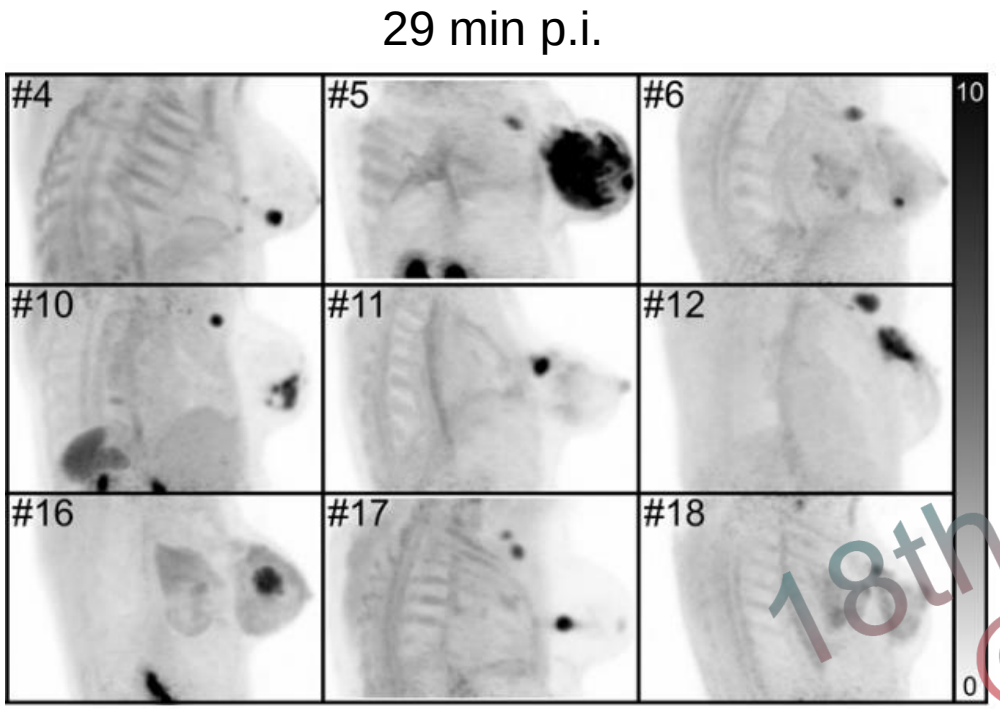
FAP-specific enzyme inhibitor (FAPI)–04

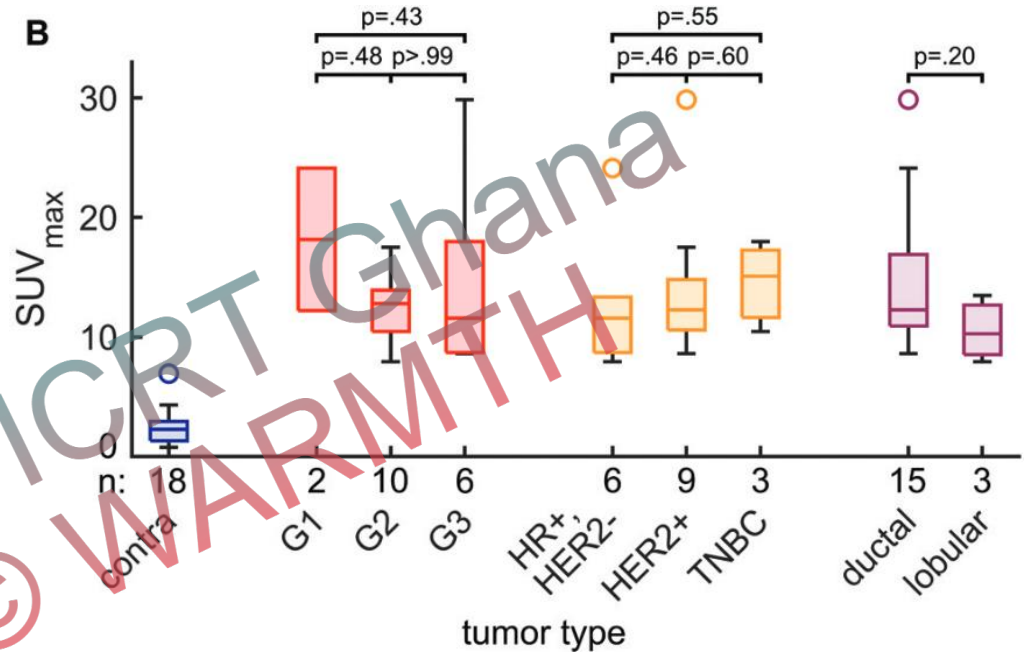


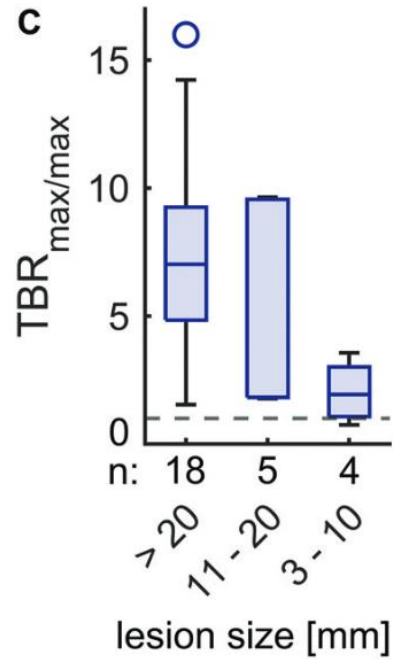
- SUV_{max} 15.3–29.9
- high-contrast images and very low uptake in normal tissue
- therapeutic treatment resulted in a significant reduction in pain medication with no side effects

Lindner et al. (2018) JNM

^{68}Ga-FAPI-46





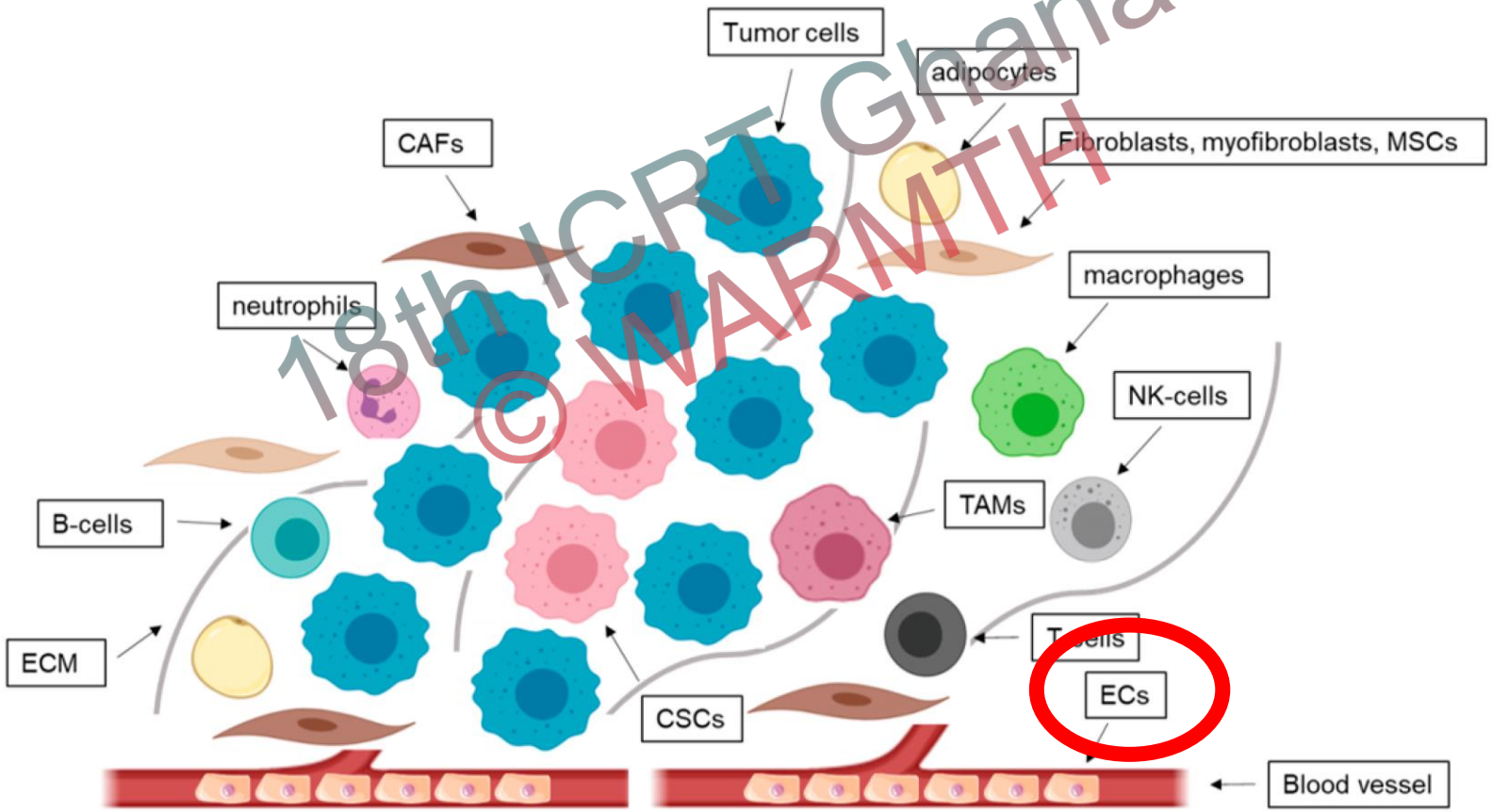


- All 19 women showed strong and reliable accumulation in invasive cancer
- mean SUVmax = 13.9 provided contrast even for the detection of subcentimeter lesions
- strong uptake of main lesions was independent between different grades, receptor status, or histological types

Backhaus et al. (2022) Radiology

Tumor Microenvironment

- PSMA expression in endothelial and epithelial cells of TNBC
- Higher PSMA expression correlated with higher grade, NST subtype, hormone receptor-negative, HER2 positive, and TNBC tumors



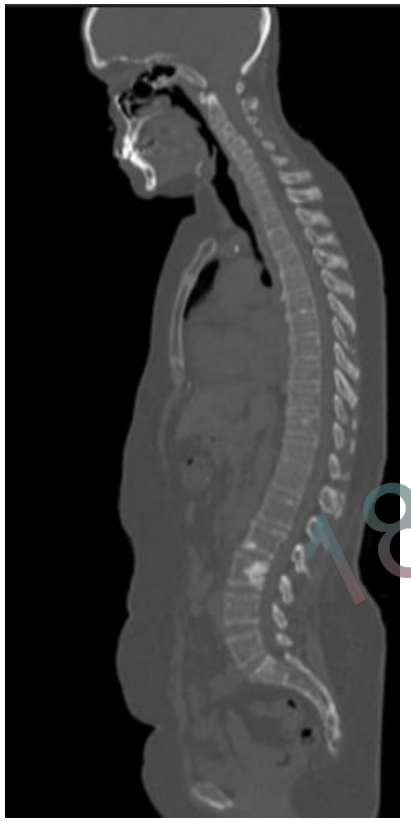
Heesch et al. (2020) Cells

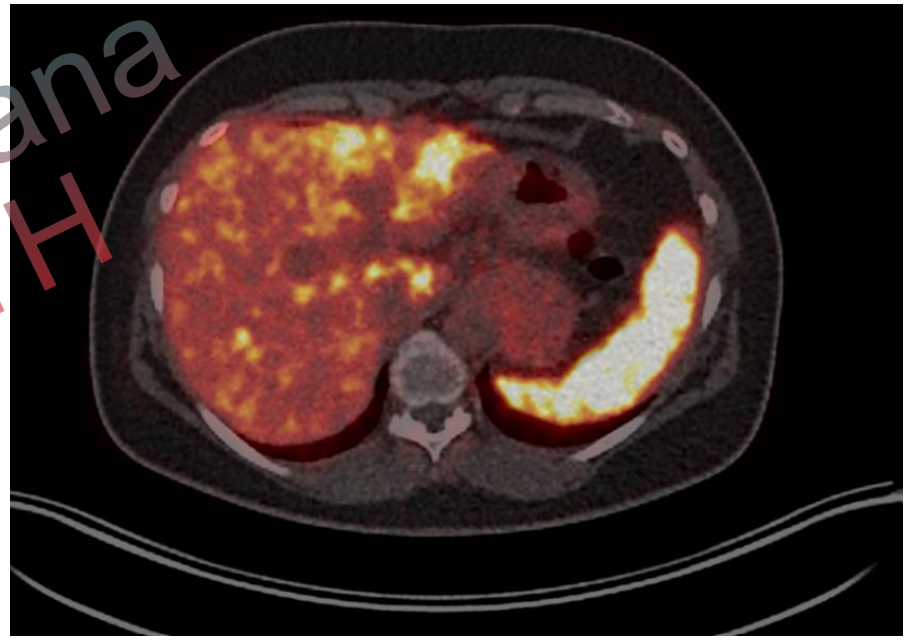
^{68}Ga-PSMA-HBED-CC

- Skeletal uptake with liver metastases

Sathekge et al. (2015) EJNMMI

^{68}Ga-PSMA

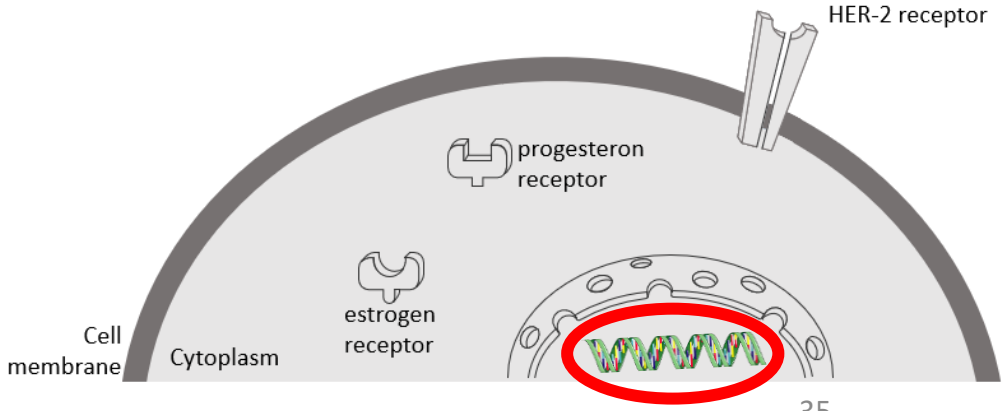





ER+, PR+, HER2-
Metastatic lesion (liver) is progredient under third-line therapy

UKA Aachen (unpublished data)

Imaging Probes for TNBC

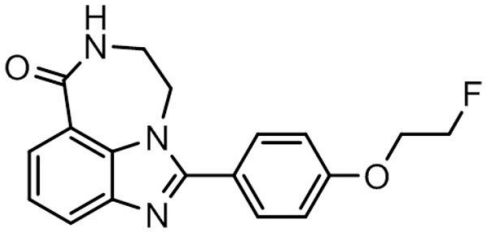


TNBC

- 15% of all BC
- Very aggressive
- Significantly earlier age of onset
- At the time of diagnosis, the tumor is larger in size and higher-graded
- Tends to develop more frequently LN, lung and brain metastasis

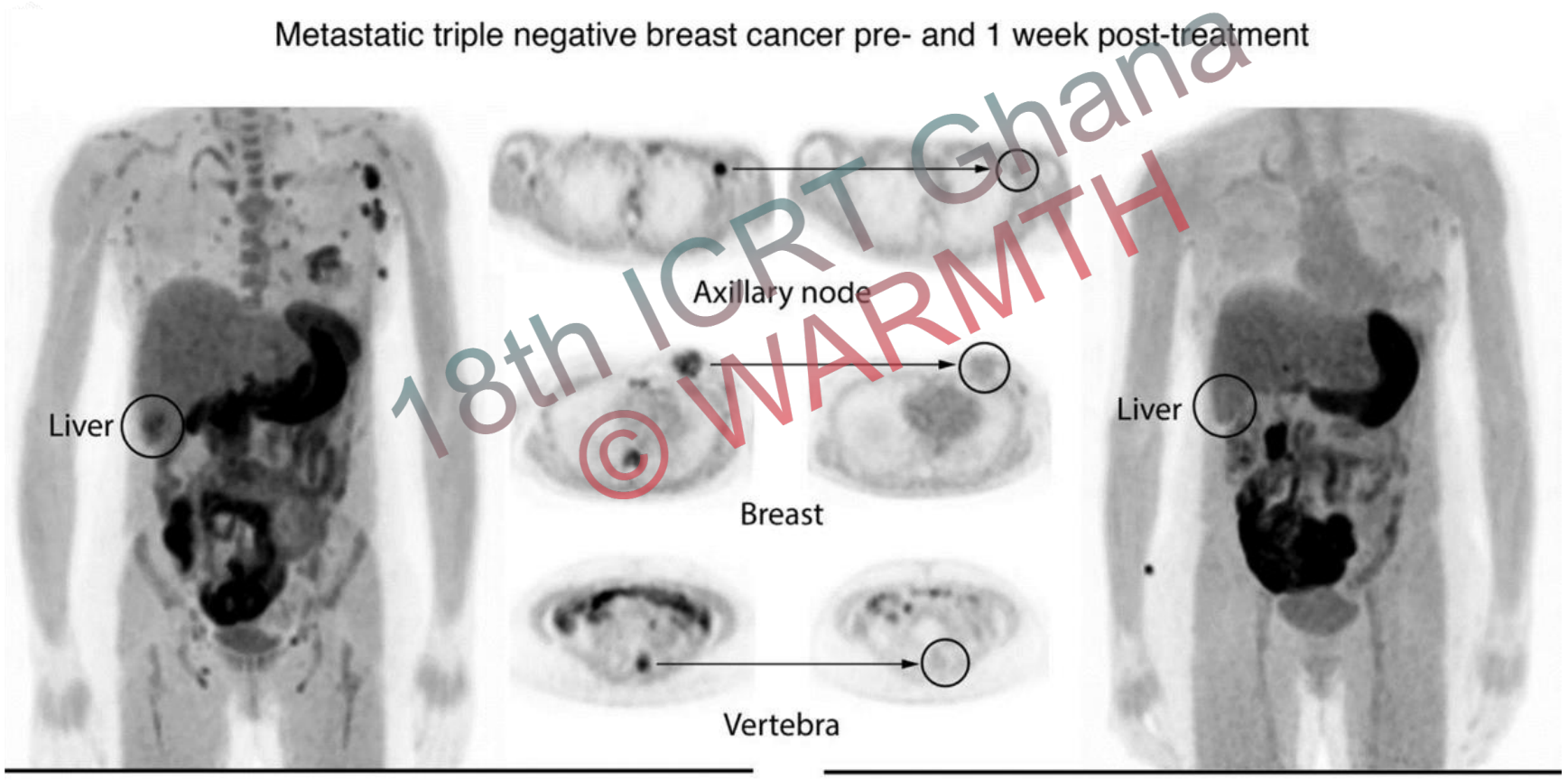
Heesch et al. (2020) Cells

[^{18}F]FluorThanatrace ([^{18}F]FTT)



[^{18}F]FTT

- Mutated BRCA is associated with sensitivity to PARP inhibition



<u>Study design</u>
n = 4
stage III and IV
3 TNBC
1 ER+

McDonald et al. (2021) JCI Insight

Summary

- for each breast cancer type, there is at least one promising tool for in vivo visualization of the molecular character:
  - ER → [^{18}F]-FES
  - PR →[^{18}F]FFNP
  - HER2 → radiolabelled nanobodies and affibodies
  - TNBC → [^{18}F]FTT
- images provide different information, and we have to bring them together to accurately determine the diagnosis in an individual case
- Two scans can work complementary to demonstrate heterogeneity of individual lesions in a single patient

UNIKLINIK RWTH AACHEN

Klinik für Nuklearmedizin

CIO

Centrum für Integrierte Onkologie
Aachen Bonn Köln Düsseldorf

Thank You!

LinkedIn

Betül Altunay

baltunay@ukaachen.de

University Hospital RTWH Aachen, Germany