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HER2-receptor quantification in breast cancer patients by imaging with ABY-025 Affibody and PET

ALI ALHUSEINALKHUDHUR

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Abstract
Alhuseinalkhudhur, A. 2024. HER2-receptor quantification in breast cancer patients by imaging with ABY-025 Affibody and PET. *Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine* 2004. 55 pp. Uppsala: Acta Universitatis Upsaliensis. ISBN 978-91-513-1995-7.

Breast cancer is the most common malignancy in women worldwide. Human epidermal growth factor receptor type 2 (HER2) is overexpressed in up to 20% of breast cancer cases and is considered an important prognostic factor and a therapeutic target. With the introduction of HER2-targeted therapy, it was important to recognize patients who will likely benefit from such treatment. Immunohistochemistry staining performed on a tumor biopsy, with in situ hybridization to detect gene amplification if needed, is the current gold standard method for HER2 receptor quantification. However, in cases with multiple metastases, it is both unfeasible and impractical to perform multiple biopsies without risking higher morbidity. Molecular imaging with tracers specifically targeting HER2 receptors provides a non-invasive approach, which allows full body quantification without the serious side effects associated with invasive biopsies. The molecule of focus in this thesis work is Affibody $Z_{HER2:2891}$ (ABY-025) molecule that has a high affinity and selectivity towards HER2 receptors.

This thesis is based on four original articles. The first part focused on the aspect of breast cancer imaging using HER2-targeting gallium-labeled tracer ^{68}Ga-ABY-025 in positron emission tomography (PET) and its role in predicting breast cancer outcome. The second part was to investigate the effect of different risk factors on developing brain metastasis, the overall survival and the effect of HER2-targeted treatment on breast cancer brain metastasis based on Uppsala County cancer registry.

We demonstrated that HER2-binding Affibody PET kinetics can be explained using a two-tissue compartment model and SUV values correlated well with the influx rates calculated using kinetic modeling, supporting its use to measure actual HER2 receptor binding. Phase II study demonstrated the potential of ^{68}Ga-ABY-025 PET to predict the treatment outcome more accurately compared to biopsy HER2-status that uses the traditional immunohistochemistry staining and in situ hybridization techniques. ^{68}Ga-ABY-025 PET provided accurate staging and reduced false positive ^{18}F-FDG PET results in HER2-positive cases. HER2-positive molecular subtypes were associated with an increased risk of developing brain metastasis. Yet, longer survival times were observed in HER2-positive subtypes receiving HER2-targeted therapy.

Keywords: PET, Breast cancer, HER2, Affibody, ABY-025, Molecular imaging, Kinetic modelling, Brain metastasis

Ali Alhuseinalkhudhur, Department of Immunology, Genetics and Pathology, Rudbecklaboratoriet, Uppsala University, SE-751 85 Uppsala, Sweden.

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وَمَا تَوْفِيقِي إِلَّا بِاللَّهِ

To Aseel, Meena, and Layan

List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

I. Alhuseinalkhudhur, A., Lubberink, M., Lindman, H., Tolmachev, V., Frejd, F. Y., Feldwisch, J., Velikyan, I., Sörensen, J. (2020). Kinetic analysis of HER2-binding ABY-025 affibody molecule using dynamic PET in patients with metastatic breast cancer. *EJNMMI Research*, 10(1):21

II. Alhuseinalkhudhur, A., Lindman, H., Liss, P., Sundin, T., Frejd, F. Y., Hartman, J., Iyer, V., Feldwisch, J., Lubberink, M., Rönnlund, C., Tolmachev, V., Velikyan, I., Sörensen, J. (2023). Human Epidermal Growth Factor Receptor 2-Targeting [^{68}Ga]Ga-ABY-025 PET/CT Predicts Early Metabolic Response in Metastatic Breast Cancer. *Journal of Nuclear Medicine*, 64(9):1364–1370

III. Alhuseinalkhudhur, A., Lindman, H., Liss, P., Sundin, T., Frejd, F Y., Feldwisch, J., Iyer, V., Lubberink, M., Velikyan, I., Sörensen, J. ^{68}Ga-ABY-025 PET in HER2-positive breast cancer: assessment of small axillary lesions - *Manuscript*

IV. Alhuseinalkhudhur, A., Sörensen, J., Jernling, M., Schiza, A., Lindman, H. Overall survival amongst patients with breast cancer brain metastasis: A cohort study based on Uppsala county cancer registry – *Submitted to British Journal of Cancer (BJC)*

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Other papers not included in the thesis:

1. Roodakker, K. R., Alhuseinalkhudhur, A., Al-Jaff, M., Georganaki, M., Zetterling, M., Berntsson, S. G., Danfors, T., Strand, R., Edqvist, P. H., Dimberg, A., Larsson, E. M., Smits, A. (2019). Region-by-region analysis of PET, MRI, and histology in en bloc-resected oligodendrogliomas reveals intra-tumoral heterogeneity. *European Journal of Nuclear Medicine and Molecular Imaging*, 46(3):569–579

2. Lindström, E., Velikyan, I., Regula, N., Alhuseinalkhudhur, A., Sundin, A., Sörensen, J., Lubberink, M. (2019). Regularized reconstruction of digital time-of-flight ^{68}Ga-PSMA-11 PET/CT for the detection of recurrent disease in prostate cancer patients. *Theranostics*, 9(12):3476–3484

Contents

Abbreviations

2TC-3k	Irreversible two-tissue compartment model
ASCO	American Society of Clinical Oncology
BBB	Blood brain barrier
BCBM	Breast cancer brain metastasis
BM	Brain metastasis
CAP	College of American Pathologists
CT	Computed tomography
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
ER	Estrogen receptor
FDA	Food and drug administration
FDG	Fluorodeoxyglucose
HER2	Human epidermal growth factor receptor type 2
HzR	Hazard ratio
IHC	Immunohistochemistry
ISH	In situ hybridization
keV	Kilo-electronvolt
MBC	Metastatic breast cancer
MRI	Magnetic resonance imaging
PBC	Primary breast cancer
PET	Positron emission tomography
PR	Progesterone receptor
ROC	Receiver operating characteristic
SPECT	Single-photon emission computed tomography
SUV	Standardized uptake value
TAC	Time activity curve
TLG	Total lesion glycolysis
VOI	Volume of interest

Introduction

Background

Breast cancer is the leading cause of cancer incidence and the fifth leading cause of cancer-related mortality worldwide, with an estimated 2.3 million new breast cancer cases in 2020.[1] In Sweden, 8619 women were newly diagnosed with breast cancer and 1326 died as a direct consequence of breast cancer in 2021.[2]

Several factors have contributed to the constant rise in incidence rates in North America and Europe over the last decades. Between 5-10 % of breast cancer cases are due to hereditary factors, among them, only a few have been recognized with gene mutations linked to breast cancer. On the other hand, there is a strong correlation between risk of developing breast cancer and hormonal factors such as early menarche, late menopause, less number of children, less breastfeeding, later age at first pregnancy, menopausal hormonal therapy, and oral contraceptives. In addition, lifestyle factors such as increased weight, alcohol consumption, and decreased physical activity were also found to be linked to increased breast cancer risk.[3,4]

Despite the increasing incidence, mortality rates in developed countries are declining (Figure 1). This could be due to early detection as a result of wider screening campaigns as well as advancements in targeted therapies.[5] In less developed countries, the mortality rates are still relatively high. This is likely due to the scarcity of the most recent diagnostic and therapeutic developments, which require huge economic resources.[1,5,6]

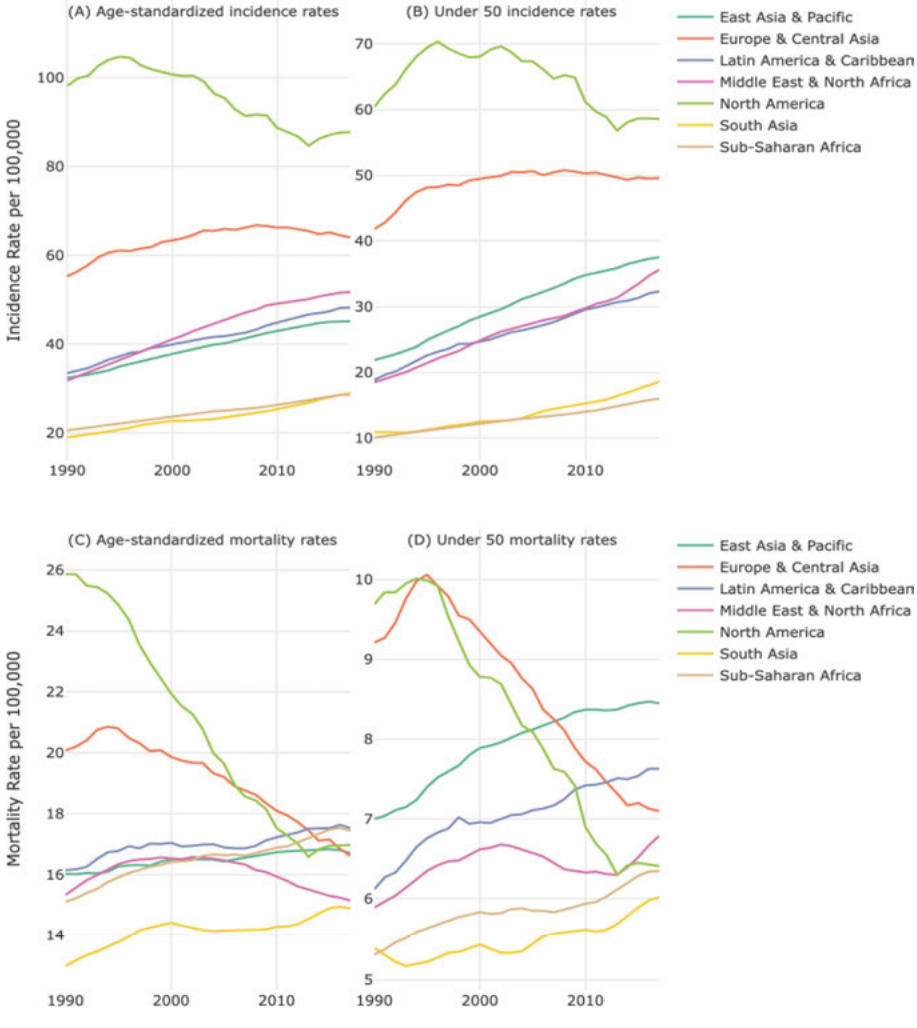

Figure 1. Breast cancer incidence and mortality rates, age-standardized (all ages) and in women under 50 years, by world region. (A) Age-standardized incidence rates for all ages. (B) Incidence rates for women under 50. (C) Age-standardized mortality rates for all ages. (D) Mortality rates for women under 50 (reprinted from Lima et al. 2021).[5]

Classification

Breast cancer is considered a group of heterogeneous diseases with variable morphological and biological differences that directly influence the clinical outcome and response to treatment. Evidence has shown that histological grade and type have an important prognostic value that helps predict tumor behavior and facilitate treatment decision.[7,8] Histological grade reflects the de-

gree of aggressiveness of the tumor cells by measuring the degree of differentiation and the mitotic index of the cells, while histological type defines the growth pattern of the cells based on their histological appearance. In the presence of many variables in breast cancer types, it is crucial to find a clinically meaningful classification system that aids oncological decision.

Breast cancer can be generally divided into non-invasive carcinoma (in situ) and invasive carcinoma, each of them can be further classified based on the cytological appearance and growth pattern into mainly ductal and lobular carcinoma. Up to 80% of invasive breast cancer cells arise from the ductal epithelium and are called invasive ductal carcinomas, whereas lobular carcinomas constitute around 10% of all invasive carcinomas. Other types include tubular, medullary, mucinous, papillary, and other rare types.[9]

Molecular subtypes

Perou, Sørlie, and their colleagues introduced a new molecular classification system for breast cancer with their groundbreaking study in 2000.[10] Based on various gene expressions, breast cancer could be classified into five clinically relevant molecular subtypes: Luminal A, Luminal B, Human epidermal growth factor receptor type 2 (HER2) positive, basal, and normal like.

This is based on immunohistochemistry (IHC) essays, hormone receptor status, including Estrogen receptors (ER) and progesterone receptors (PR), HER2 status, and proliferation measured as Ki-67. Luminal A is defined as ER or PR positive or both, HER2-negative, low proliferation and low grade, and luminal B is defined as ER or PR positive or both, HER2-negative, and high proliferation or high grade. HER2-positive subtype can also be further divided into Hormonal receptor-positive (HER2-luminal) and hormonal receptor-negative.[9]

Human epidermal growth factor receptor type 2 (HER2)

HER2 receptor belongs to the epidermal growth factor (EGF) family of receptor tyrosine kinases together with 3 other receptors, EGFR (HER1), HER3, and HER4.[11] HER2 is normally expressed at low levels in various tissues such as breast, skin, and gastrointestinal tract. Its function is believed to mediate cell-to-cell interactions necessary for cell growth and proliferation as well as cell differentiation.[12,13] When it comes to HER2-positive cancer cells, the number of gene copies is amplified, which results in overexpression of HER2 receptors with up to a 100-fold increase in density.[14] HER2 receptors have no natural ligand, instead, they get activated via homo- or heterodimerization with other members of HER-receptor family, which triggers a series of down-

stream signaling known to aggravate malignant properties in cancer cells (Figure 2).[15] For this reason, inhibition of HER2 receptor dimerization is an attractive target to suppress tumor growth.

HER2 overexpression in cancer cells is linked to carcinogenesis and increased metastatic tendency.[12,16] Up to 20% of breast cancer cases presented with HER2-receptor overexpression with or without gene amplification. Since the late 1980s, HER2 status role in breast cancer pathogenesis and prognosis has been well-established. HER2 expression is associated with worse overall survival and shorter time to relapse in breast cancer patients, showing an even higher significance than hormonal status.[17] However, since the introduction of HER2-targeted antibodies such as trastuzumab in 1998, the outcome of the disease has been dramatically improved.[18,19]

Systemic treatment in breast cancer

Adjuvant therapy improves treatment outcome and reduces recurrence rates in breast cancer patients.[20,21] The choice of the treatment regimen depends heavily on the molecular subtype, the stage of the disease, and which lines of treatment the patient received previously. Early-stage breast cancer without local spread is usually treated surgically, with or without radiotherapy. However, in some cases with more aggressive or diffuse disease, or with spread to the local lymph nodes, such as axillary, supra- or infraclavicular nodes, neoadjuvant systemic treatment might be considered. The goal is to reduce the tumor size, thus maximizing the chances for curative surgical resection and reducing the risk of recurrence.[22]

Systemic therapy is usually given as chemotherapy with either anthracyclines, taxanes, or both. Platinum-based chemotherapy can also be considered as a second line of treatment and is proven effective in triple-negative breast cancer.[23] If the patient has HER2-positive disease, HER2-targeted monoclonal antibodies such as trastuzumab and pertuzumab can be added. For triple-negative cases, immunotherapy in the form of pembrolizumab can be added to the neoadjuvant regimen, then as adjuvant therapy after surgery.[24,25]

With local recurrence, systemic therapy is given after surgical excision with a curative intent. Treatment in the metastatic setting is mainly palliative. The presence of biological targets such as the expression of certain hormonal receptors on cancer cells allows for endocrine therapy, such as tamoxifen and aromatase inhibitors, which are much more tolerable than cytostatic drugs that are associated with more serious side effects.[26,27]

Some metastatic involvements are associated with poor prognosis. Brain metastasis (BM) is usually associated with more aggressive types of breast cancer, such as HER2-positive subtypes, that require intensive treatments. While in luminal breast cancer, BM is less common and tends to develop at a later stage of the disease. Systemic chemotherapy has shown limited effect in

the treatment of BM as most chemotherapy agents cannot cross the blood-brain barrier (BBB). However, in HER2-positive disease with BM, HER2-targeted antibodies, such as trastuzumab, and tyrosine kinase inhibitors, such as lapatinib, in combination with other drugs have shown evidence in preventing the development of BM as well as being effective in prolonging the overall survival.[28–30]

HER2-targeted treatment

HER2-targeted treatments, such as humanized monoclonal antibodies trastuzumab and pertuzumab, have changed the outcome of HER2-positive breast cancer drastically. Trastuzumab was approved by the U.S. Food and Drug Administration (FDA) in 1998 for treatment-naïve, HER2-positive breast cancer patients in combination with chemotherapy. Later in 2006, the FDA approved its use for the adjuvant treatment of surgically removed HER2-positive breast cancer. Lastly, in 2010, it was approved in HER2-positive gastric tumors.[19,31,32]

Trastuzumab binds to the extracellular domain IV of HER2 receptor (Figure 2), thus inhibiting cell growth by blocking the intracellular signaling system.[33] It has also been shown to suppress cellular repair when cells are exposed to radio- or chemotherapy and promote cell apoptosis.[34,35]

The success of trastuzumab story encouraged researchers to examine other antibodies, one of which is pertuzumab. Pertuzumab has a different mechanism of action than trastuzumab. While trastuzumab prevents HER2 receptor dimerization with another HER2 receptor, pertuzumab acts by preventing HER2 dimerization with HER3 receptors. When used in combination, trastuzumab and pertuzumab have a significantly better outcome than trastuzumab alone.[24,36,37]

The American Society of Clinical Oncology and College of American Pathologists (ASCO/CAP) guidelines state that IHC staining with or without in situ hybridization (ISH) should be performed on at least one biopsy specimen taken from the primary tumor and if possible one metastatic breast cancer (MBC) lesion to aid the decision to give HER2-targeted treatment.[38,39] However, biopsy is not always feasible, especially in the metastatic setting. Breast cancer is a heterogeneous disease and variations in HER2 receptor expression across lesions within the same patient have been reported.[40,41] In addition, some tumors have shown resistance to HER2-targeted treatment, despite their expression of HER2.[42] All of this poses a challenge to proper stratification of patients that will most likely benefit from receiving HER2-targeted treatment.

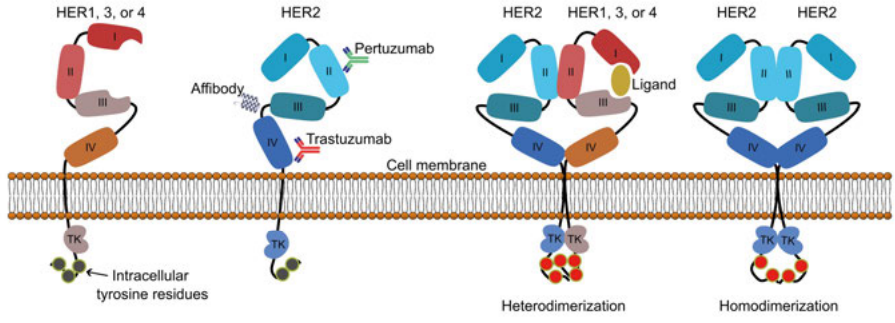

Figure 2. Human epidermal growth factor receptor (HER) on cell surface. HER2 receptor gets activated via hetero- (ligand activates other members of HER family) or homodimerization (HER2/HER2), which leads to phosphorylation, and initiates downstream intracellular signaling. Affibody molecules bind to a different extracellular site (The junction between domain III and IV) than Trastuzumab and Pertuzumab (Domain IV and domain II, respectively). TK = tyrosine kinase

HER2-targeted therapy resistance

Despite the clinical success achieved with the use of trastuzumab, resistance to HER2-targeted therapy remains a clinical challenge as a significant number of adjuvant and a greater number of recurrent breast cancer patients do not respond to trastuzumab treatment.[31,43–45] Both initial and acquired resistance mechanisms against HER2-targeted drugs such as trastuzumab have been proposed. Some mechanisms are responsible for the initial resistance to HER2-targeted therapy, such as a truncated HER2 receptor that blocks access of trastuzumab to its binding site on the extracellular domain, or alterations in the intracellular signaling pathways. For acquired resistance, mechanisms such as upregulations of other HER receptors and their ligands, or deregulations in target signaling pathways have been described.[46–49]

As an effort to overcome both initial and acquired resistance mechanisms and improve efficacy, trastuzumab is usually given in combination with other drugs. For example, Ado-trastuzumab Emtansine, which is an antibody-drug conjugate, has improved treatment outcome in HER2-positive breast cancer.[50] In other instances, trastuzumab is given in combination with other antibodies such as pertuzumab, which has a different binding site on HER2 receptor (extracellular domain II), or with tyrosine kinase inhibitors such as lapatinib. These combination therapies have a better response to treatment than monotherapy with trastuzumab only.[51,52]

Imaging in breast cancer

Imaging improves the discovery of otherwise clinically undetectable malignant lesions and gives a global overview of cancer spread. Besides, image-guided biopsy helps obtain tissue samples from metastatic lesions that are otherwise not feasible. Imaging techniques such as mammography, ultrasonography, computed tomography (CT) and magnetic resonance imaging (MRI) have been routinely used in the clinic for the diagnosis, staging, and treatment follow-up of cancer patients.[53] While functional imaging in the form of single-photon emission computed tomography (SPECT) or positron emission tomography (PET) is used in measuring the biological processes on the molecular level.

Molecular Imaging

The most widely used molecular imaging tracer by far is the glucose analogue 2-[^{18}F]fluoro-2-deoxy-d-glucose (^{18}F-FDG PET), which is an effective tool in detecting distant metastasis, recurrent disease, and in measuring treatment response.[54,55] ^{18}F-FDG PET measures the metabolic activity of tissues, which tends to be higher in cancer cells due to their rapid growth and high mitotic activity. However, this phenomenon is not specific to cancer cells as there are other non-malignant conditions with high metabolic activity, which means that these conditions will also appear positive on ^{18}F-FDG PET.[56,57] Moreover, it is not possible to validate the expression of certain biomarkers such as HER2 receptors with ^{18}F-FDG PET.

HER2 receptor overexpression as mentioned earlier is an important biomarker and its quantification is essential in treatment decision. This prompts researchers to pursue more specific tracer alternatives that will facilitate a whole-body evaluation of the HER2-receptor expression. Several probes have been investigated using SPECT and PET including, among others, antibodies, antibody fragments, and affibodies.[58–61] ^{64}Cu-trastuzumab and ^{89}Zr-trastuzumab tracers were investigated as imaging probes for HER2-expression in MBC.[62,63] ^{64}Cu-trastuzumab uptake in tumor tissue showed a strong correlation with the patient HER2 status confirmed by biopsy, while ^{89}Zr-trastuzumab in combination with ^{18}F-FDG PET showed a predictive value in patients receiving HER2-targeted treatment. Despite the promising results, antibodies are relatively large molecules and have slow pharmacokinetics, which means image acquisition is usually performed 1 to 4 days after injecting the tracer.

Affibody molecules

Affibodies are small molecules (58 amino acids, 7 kDa) that have a high affinity towards HER2 receptors and are much smaller than antibodies, thus have faster pharmacokinetics. This translates to a lesser time required for blood clearance and higher tumor-to-blood ratios, resulting in a much shorter waiting time after tracer injection that allows acquisition within a few hours.[59] One of the Affibody molecules that showed promising results is the Affibody $Z_{HER2:2891}$ (ABY-025) molecule.[60,64] Labeled with the positron-emitting radioisotope Gallium 68 (^{68}Ga), it was possible to achieve PET images with good contrast after only 2 hours of injection in patients with advanced breast cancer.[61]

Basic principles in PET

PET is a functional imaging technique that in its basic principle relies on the annihilation phenomena of positrons emitted by certain isotopes. A positron, which has the same mass as an electron but carries a positive charge, travels very small distances within a few millimeters. When a positron loses most of its kinetic energy, it collides with an electron in what is known as annihilation process. Each annihilation process produces two photons carrying an energy of 511 keV and travel in opposite directions. PET camera contains multiple detectors arranged in a ring that can coincidentally detect these two photons and estimate the line of response between the two points (Figure 3). With the help of a CT image to calculate attenuation factors, an image can be reconstructed from multiple measurements of radioactivity.[65,66]

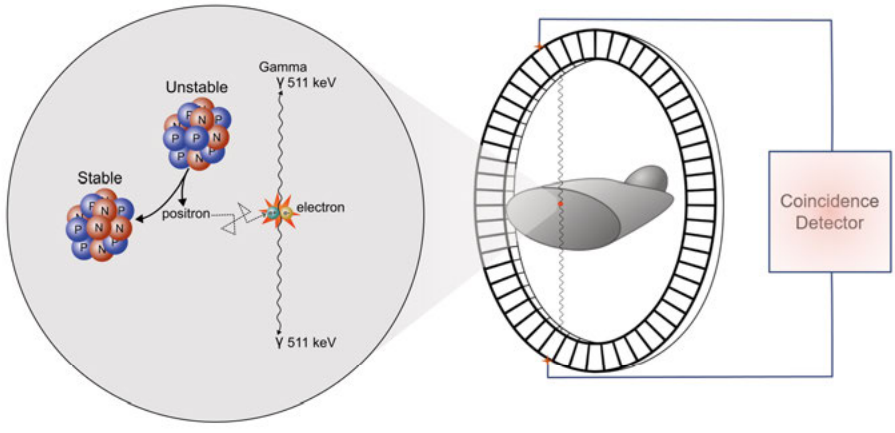

Figure 3. Schematic illustration of the basic principles of PET. Positron-emitting isotopes emit a positively charged positron that annihilates when met with an electron, producing two photons traveling in opposite directions carrying the same energy (511 keV). These two coincidence photons are then detected by a PET camera.

Quantification in PET

PET measures the activity concentration per unit volume in tissue. Activity concentration measurements vary based on the injected activity and patient's weight. These variations make it difficult to compare between different PET scans. One way to overcome this issue is to use a standardized method, such as standardized uptake value (SUV). SUV is a method to normalize the activity concentration to the injected dose and body weight. While it is a semi-quantitative method that can be easily acquired with a simple formula, it is not completely independent from certain patient parameters such as body size and composition, and scanner parameters such as dose calibration.[67,68]

In nuclear medicine, the reduction in ^{18}F-FDG uptake in a specific region after treatment can be used as a means to measure the response rate to treatment, this facilitates the evaluation of multiple lesions in the same patient without the need for multiple biopsies.[54]

Kinetic modeling

Tumor uptake in PET is routinely reported as SUV. While it is generally acceptable in most situations, SUV values do not necessarily reflect the true kinetics of the tracer, especially in the case of ligand-receptor binding. The estimation of the influx rate (K_i) to a certain tissue on the other hand can accurately reflect the actual binding of the tracer to the target, for example HER2

receptor. This provides a method to validate the use of SUV as a representative value to the actual receptor binding, especially for new tracers.

In dynamic PET scans, data is collected sequentially after the injection of the tracer, enabling the measurement of activity concentration in certain tissues over time. The measured time-activity curves (TACs) can then be used, with the help of compartmental modeling or graphical analysis, to calculate the kinetic parameters of the tracer. In compartmental modeling, it is assumed that there are separate pools of tracer activity that are called compartments. For example, a one-tissue compartment, which is the simplest, is usually used to estimate the kinetics of tracers aimed at measuring the blood flow in tissues. Another example is the two-tissue compartment model, more suitable for ^{18}F-FDG and receptor-ligand interactions. Here, the tracer moves from the arterial blood compartment to the free tissue compartment, and then the tracer moves to the second tissue compartment where it becomes specifically bound.[69]

The estimation of K_i can be achieved either by performing a voxel-based analysis through creating parametric images or by running a Volume-of-interest (VOI)-based analysis, using TACs as an input function to estimate the kinetic parameters (Figure 4). Graphical analysis techniques, such as Patlak plot, use linear regression to calculate the kinetic parameters with the assumption of tracer irreversibility.[70,71]

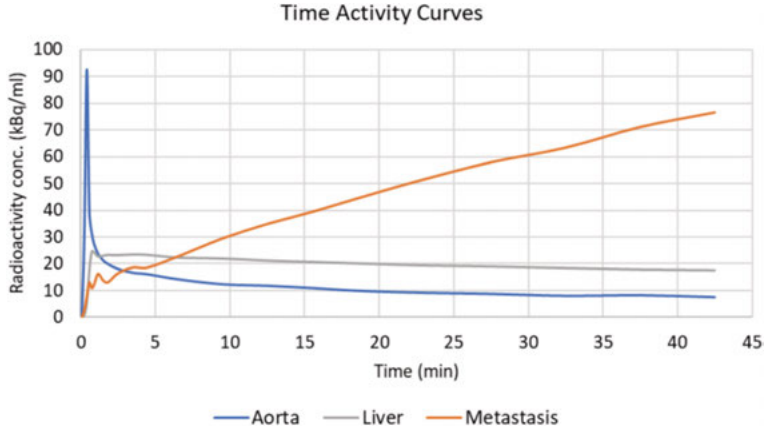

Figure 4. Time activity curves based on VOI-based analysis of ^{68}Ga-ABY-025 dynamic PET scan in a breast cancer patient with liver metastasis.

Aims of the thesis work

The overall thesis aims are to evaluate the role of the ^{68}Ga-ABY-025 PET as a quantitative tool for the determination of HER2 expression and prediction of treatment response in patients with breast cancer and to explore factors influencing the risk and overall survival of brain metastasis in breast cancer patients.

Specific aim for each paper:

Paper I

To explore the applicability of kinetic modeling and parametric image analysis for absolute quantification of ^{68}Ga-ABY-025 uptake and HER2-receptor expression and how that relates to static SUVs.

Paper II

To investigate how ^{68}Ga-ABY-025 uptake correlates to biopsy-based HER2 status and to predict HER2-targeted treatment outcome.

Paper III

To assess the different pathological and physiological uptake patterns in both treatment-naïve and recurrent HER2-positive breast cancer patients.

Paper IV

To investigate the risk factors of developing BM in MBC and estimate the overall survival based on breast cancer molecular subtypes.

Patients and methods

Study populations

In paper I, we reanalyzed 16 patients with MBC from an earlier study (Table 1).[61] Based on their IHC and ISH results, 12 out of 16 were HER2-positive while 4 were HER2-negative. Thirteen patients had liver metastases.

Paper II was a prospective single-center phase II diagnostic study, a part of the Affibody-3 clinical trial (NCT03655353). Forty patients with biopsy-confirmed HER2-positive breast cancer were included with either stage II or III breast cancer planned for neoadjuvant therapy or recurrent MBC.

In paper III, the 40 patients included in paper II in addition to another 10 patients recruited as part of the Affibody-3 clinical trial were analyzed.

Paper IV was a retrospective observational study based on data from a real-time treatment registry (RealQ®) and included 663 patients with MBC treated at Uppsala University Hospital between 2009 and 2022.

Production of ^{68}Ga-ABY-025

Vials containing GMP-quality ABY-025 peptide were provided by Affibody AB. In Paper I, the labeling process resulted in a peptide dose product of 427 ± 19 μg with the use of a ^{68}Ge/^{68}Ga generator (1850 MBq IGG100).

In paper II and III, the production of ^{68}Ga-ABY-025 was done using a fully automated system, yielding a 98% ± 1% radiochemical purity (peptide dose of 327 ± 29 μg).

^{18}F-FDG PET

Patients in paper I, II, and III all underwent ^{18}F-FDG PET at baseline (GE Discovery ST/MI, GE healthcare). In paper II and III, a follow-up ^{18}F-FDG PET was performed after 2 cycles of treatment to assess metabolic tumor response. The scans were performed according to a standard clinical protocol 1 h after the injection of 3 MBq of ^{18}F-FDG per kilogram body weight.

^{68}Ga-ABY-025 PET

In paper I, all patients underwent simultaneous 45-minute dynamic ^{68}Ga-ABY-025 PET (22 frames; 6 × 10, 3 × 20, 3 × 60, 5 × 180, 5 × 300 seconds) over the upper abdomen with ^{68}Ga-ABY-025 tracer injection (241 ± 49 MBq). In two patients, dynamic scans were performed over the chest region instead, where the lesions were located. Static PET scans were acquired 2 h and 4 h post-injection (4 and 5 min per bed position, respectively) for the first 10 patients (group 1), and 2 h post-injection for the last 6 patients (group 2). Five patients in group 2 underwent repeat scans (retest) the following week to investigate reproducibility of tracer uptake measurements. Images were reconstructed according to the vendor-supplied settings (2 iterations and 21 subsets; corrections for dead time, decay, and attenuation) into a 128 × 128 matrix with voxel size 3.9 × 3.9 × 3.27 mm.

In paper II and III, all patients underwent a baseline static ^{68}Ga-ABY-025 PET 3 h post-injection with 139 ± 43 MBq of ^{68}Ga-ABY-025 (cold peptide content = 262 μg). Acquisition time was 4 minutes per bed position.

Image analysis

Dynamic PET analysis

In paper I, volumes of interest (VOIs) were defined manually using Carimas 2.9 (Turku PET Center, Turku, Finland), and concurrent CT images were used to guide VOI definition. VOI-based kinetic analysis of dynamic PET images was performed using a MATLAB program written in-house. Among the compartmental models tested, the irreversible two-tissue compartment model (2TC-3k) (Figure 5) returned the best fit according to the Akaike information criterion.

Based on the 2TC-3k model, parametric images of tracer delivery constant (K_1) and K_i were created, with the descending aorta TACs as input function, using a MATLAB program written in-house. Parametric images of K_i based on Patlak graphical analysis were also created. The VOI-based analysis was performed to validate parametric images.

Static PET analysis

SUVs were calculated from 2 h and 4 h post-injection (paper I) or 3 h post-injection (paper II, III) static whole-body images. Carimas 2.9 (Turku PET Center, Turku, Finland) in paper I and Hermes Hybrid viewer (Hermes Medical Solutions AB, Stockholm, Sweden) in paper II and III were used for VOI definition and image analysis.

^{18}F-FDG PET was used to guide VOI definition (paper I-III) and to measure the metabolic response as change in total lesion glycolysis (Δ-TLG) (paper II, and III).

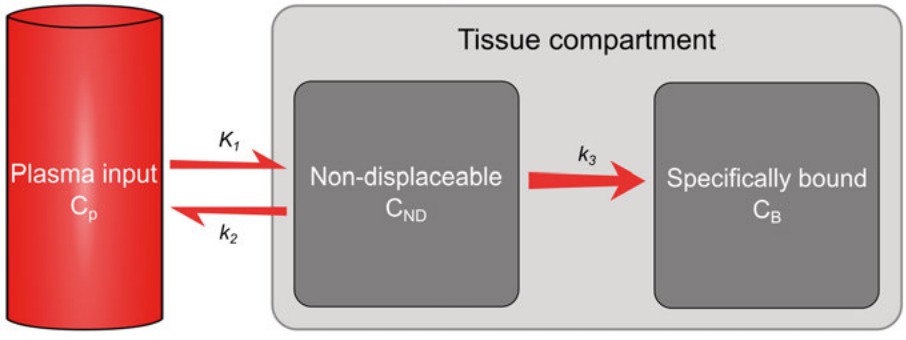

Figure 5. Irreversible two-tissue compartment kinetic model.

Statistical analysis

Nonparametric tests were used to compare different groups in paper I, Mann-Whitney test for independent comparisons, and Wilcoxon's signed-rank test for matched pairs. Bland-Altman plot and intraclass relative repeatability coefficient were calculated to investigate reproducibility in group 2 of patients who underwent retest scans.

In paper II, cutoff values to predict positive metabolic outcome were estimated using Receiver operating characteristic (ROC) curve. Multivariate analysis was used to investigate the relationship between different variables and the treatment outcome. Nonparametric analysis of variance (ANOVA) was used to investigate differences between patient groups stratified according to the number of previous treatments received. Spearman's ρ (rho) was used to investigate the correlation between the variables in paper III.

Kaplan-Meier estimator was used to calculate overall survival in paper IV, log-rank test was used to compare survival between different groups. Risk ratios between variables were calculated using Cox-proportional hazards model.

Table 1. Patient characteristics in paper I (reprinted from Sörensen et al. 2016).[61]

	HER2-positive cohort (n=12) Number of pts (%)	**HER2-negative cohort (n=4) Number of pts (%)**
Median age in years (range)	61 (33-74)	66 (41-72)
ER-positive	9 (75%)	3 (75%)
Sites of disease		
Locoregional	4 (33%)	2 (50%)
Bone	8 (67%)	3 (75%)
Liver	9 (75%)	4 (100%)
Lung	2 (17%)	1 (25%)
Lymph nodes	3 (25%)	1 (25%)
CNS	3 (25%)	2 (50%)
Contralateral breast	0	1 (25%)
Other	4 (33%)	1 (25%)
On-going trastuzumab	11 (92%)	0
Biopsy – post PET	9 (75%)	3 (75%)
Confirmed conversion of HER2 status from primary to metastasis	1 (8%)	2 (50%)

Results

Paper I

We found that, among the compartmental models we tested, the irreversible two-tissue compartment (2TC-3k) model was the best to fit the kinetics of ^{68}Ga-ABY-025, giving the lowest Akaike information criterion for the majority of TACs. Parametric K_i images and VOI-based K_i values were basically similar for both 2TC-3k model and Patlak graphical analysis (Figure 6. A, B). K_i values obtained from VOI-based analysis and parametric images were basically similar ($R^2 > 0.99$, $p < 0.001$, n=24) (figure 6. C, D). This means that both can be used interchangeably.

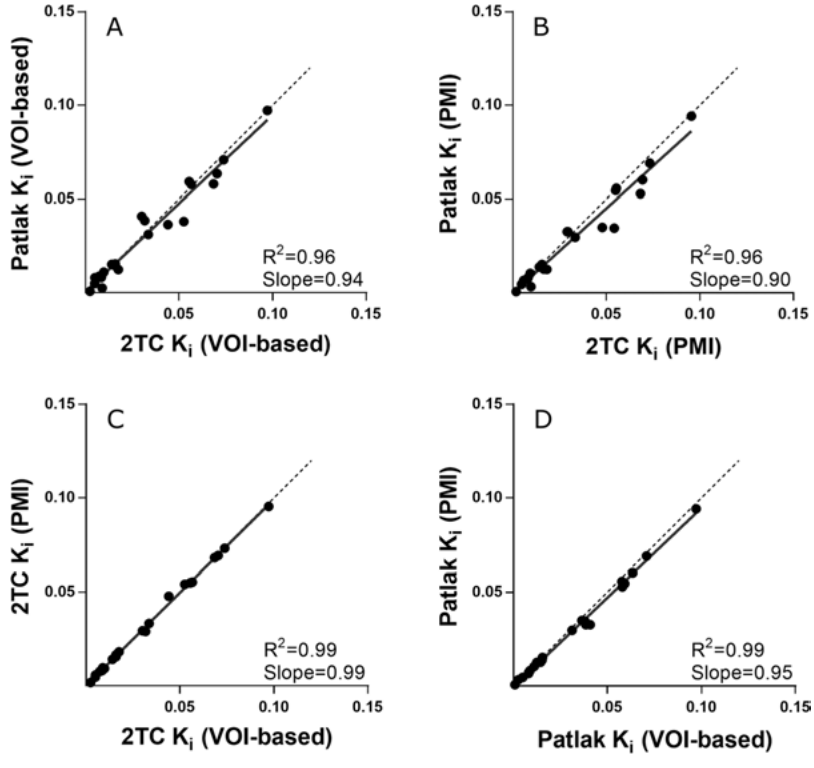

Figure 6. Correlations between **A** VOI-based 2TC and Patlak K_i's, **B** parametric 2TC and Patlak K_i's **C** 2TC K_i's VOI-based and parametric, and **D** Patlak K_i's VOI-based and parametric. PMI = parametric images

Both 2TC and Patlak Parametric K_i images showed an advantage over static SUV images by eliminating non-specific background uptake. This led to a favorable tumor-to-background ratio (TBR) of 3.7 ± 2.8 in 2TC K_i and 7.1 ± 7.8 in Patlak K_i (Figure 7). The latter was significantly higher than SUV-based TBR (4.2 ± 3.4 2 h post-injection). SUV values at 2 h and 4 h had a good correlation with K_i values both from 2TC and Patlak methods (Figure 8).

In HER2-negative metastatic lesions, both SUV and K_i values were equal or lower than the normal liver uptake (Figure 7 B). We also found that tracer delivery, presented as parametric K_1 images, was lower in the metastatic lesions compared with the normal tissue (Figure 7).

Both 2TC K_i and Patlak K_i showed comparable repeatability to static SUV images without noticeable bias.

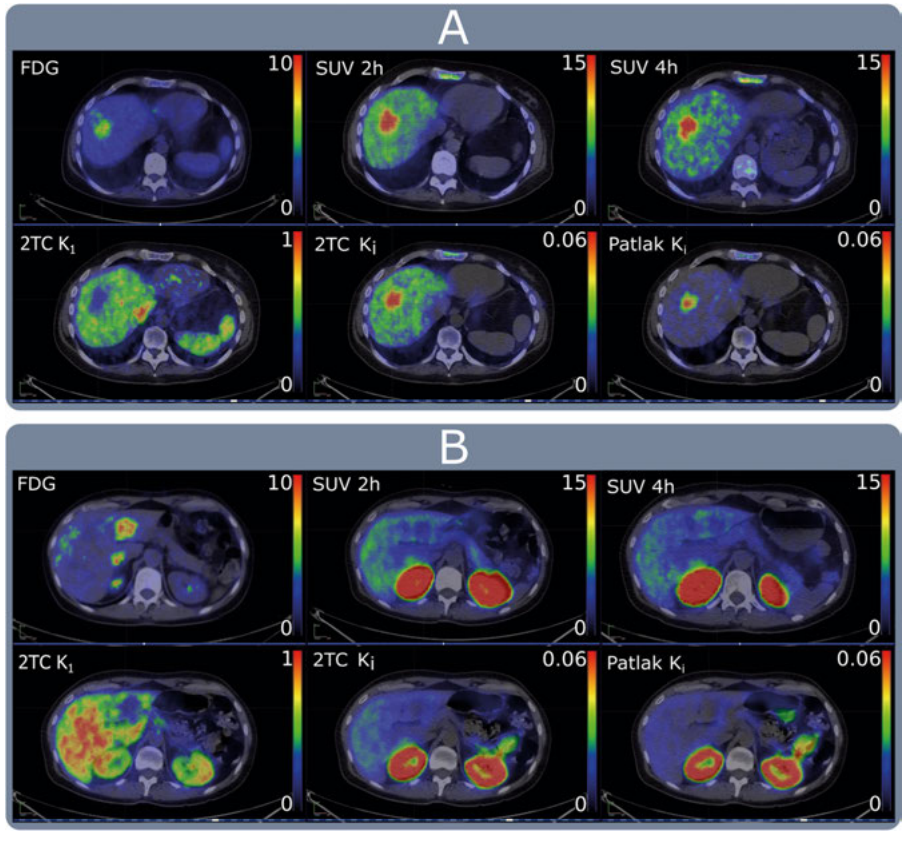

Figure 7. ^{18}F-FDG PET/CT and ^{68}Ga-ABY-025 PET/CT images and parametric images derived from dynamic PET in **A** patient with HER2-positive liver metastasis **B** patient with HER2- negative liver metastasis. K_1 = delivery rate, K_i = net uptake rate, 2TC = irreversible two-tissue compartment

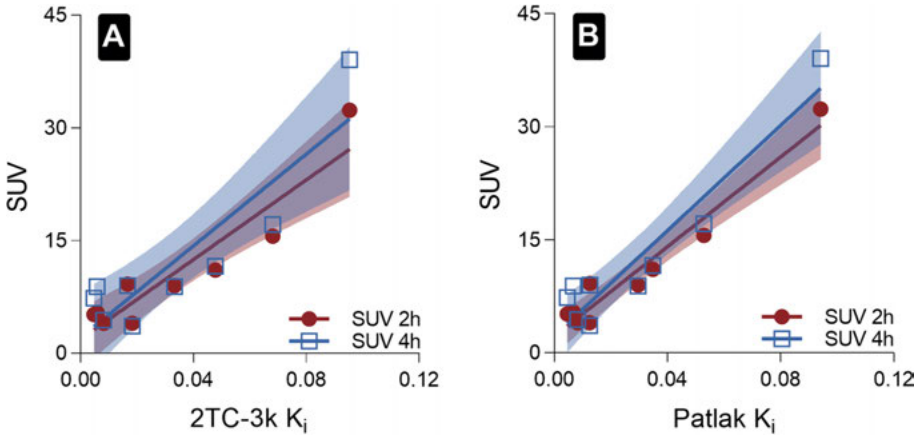

Figure 8. A 2TC-3k K_i correlation with SUV 2 h and SUV 4 h (R^2 = 0.87, 0.80 respectively, both $p < 0.0001$). **B** Patlak K_i correlation with SUV 2 h and SUV 4 h (R^2 = 0.95, 0.90 respectively, both $p < 0.0001$). Shaded areas represent 95% confidence band

Paper II

In this study, we reported the results of phase II of ^{68}Ga-ABY-025 PET prospective diagnostic clinical trial (Affibody-3). Nineteen patients with primary breast cancer (PBC) and 21 patients with MBC were included. Twenty patients out of 40 were treatment-naïve, 12 had received 1 to 3 treatments, and 8 patients had received more than 3 treatments prior to the current study. Based on biopsy results, 31 patients were HER2-positive (IHC 3+ or 2+ / ISH amplified), 3 patients were borderline HER2-positive (IHC 2+ / ISH not amplified), and 6 patients were HER2-negative (IHC 0 or 1+). Patient characteristics are summarized in table 2.

We observed heterogeneity in ^{68}Ga-ABY-025 PET uptake even at the intrapatient level (mean difference in SUVmax, 7.6; range, 1.2-39.1) (Figure 9 A and C). Using a cutoff SUVmax of 6.0 to determine HER2 positivity, ^{68}Ga-ABY-025 PET results did not match biopsy-based HER2 status in 12 patients (Figure 9 B). We did not find a significant correlation between ^{68}Ga-ABY-025 PET and biopsy-based results (p = 0.13) (Figure 10).

It was possible to predict the metabolic treatment response measured as Δ-TLG below -25% using ^{68}Ga-ABY-025 PET with a cut-off SUVmax of 10.7 in all patients (AUC, 0.61; 56% sensitivity; 66% specificity; p = 0.03), whereas biopsy-based HER2 status did not significantly predict metabolic response (AUC, 0.58; 79% sensitivity; 37% specificity; p = 0.06).

Table 2. Patient characteristics and descriptive data in paper II.

	HER2 status based on IHC and ISH results		
Item	Positive (n = 31)	Negative (n = 6)	Borderline (n = 3)
Median Age (y) (range)	57 (29-89)	63 (45-78)	58 (53-62)
ER-positive (≥10%)	14 (45%)	4 (67%)	2 (67%)
Stage			
II	14 (45%)	0	1 (33%)
III	2 (7%)	1 (17%)	0
IV	15 (48%)	5 (83%)	2 (67%)
Molecular Subtype			
Luminal-A		1 (17%)	
Luminal-B		3 (50%)	2 (67%)
HER2-pos	17 (55%)		
HER2-pos/Luminal	14 (45%)		
Triple Negative		2 (33%)	1 (33%)
Neoadjuvant Treatment			
Primary	15 (48%)	1 (17%)	
Metastatic	2 (6%)		1 (33%)
Previous treatments			
PBC			
None	17 (55%)	1 (17%)	1 (33%)
MBC			
None			1 (33%)
1	1 (3%)	1 (17%)	0
2	5 (16%)	2 (33%)	0
3	3 (10%)	0	0
4	2 (6%)	1 (17%)	0
5	1 (3%)	1 (17%)	0
6+	2 (6%)	0	1 (33%)

For soft tissue lesions in the MBC group, the previously proposed cutoff SUVmax of 6.0 showed 86% sensitivity and 67% specificity (AUC, 0.74; 95% CI, 0.67–0.82; $p = 0.01$). For skeletal lesions, a higher cutoff SUVmax of 16.2 predicted the metabolic response (AUC, 0.81; 95% CI, 0.74–0.87; 69% sensitivity; 83% specificity; $p = 0.003$). We did not find a significant predictive value for Biopsy-based HER2 status in either soft tissue lesions or skeletal lesions.

All PBC patients responded to treatment with a variable degree. We could not find a significant predictive value for either ^{68}Ga-ABY-025 PET or biopsy-based HER2 status for metabolic response in the PBC group.

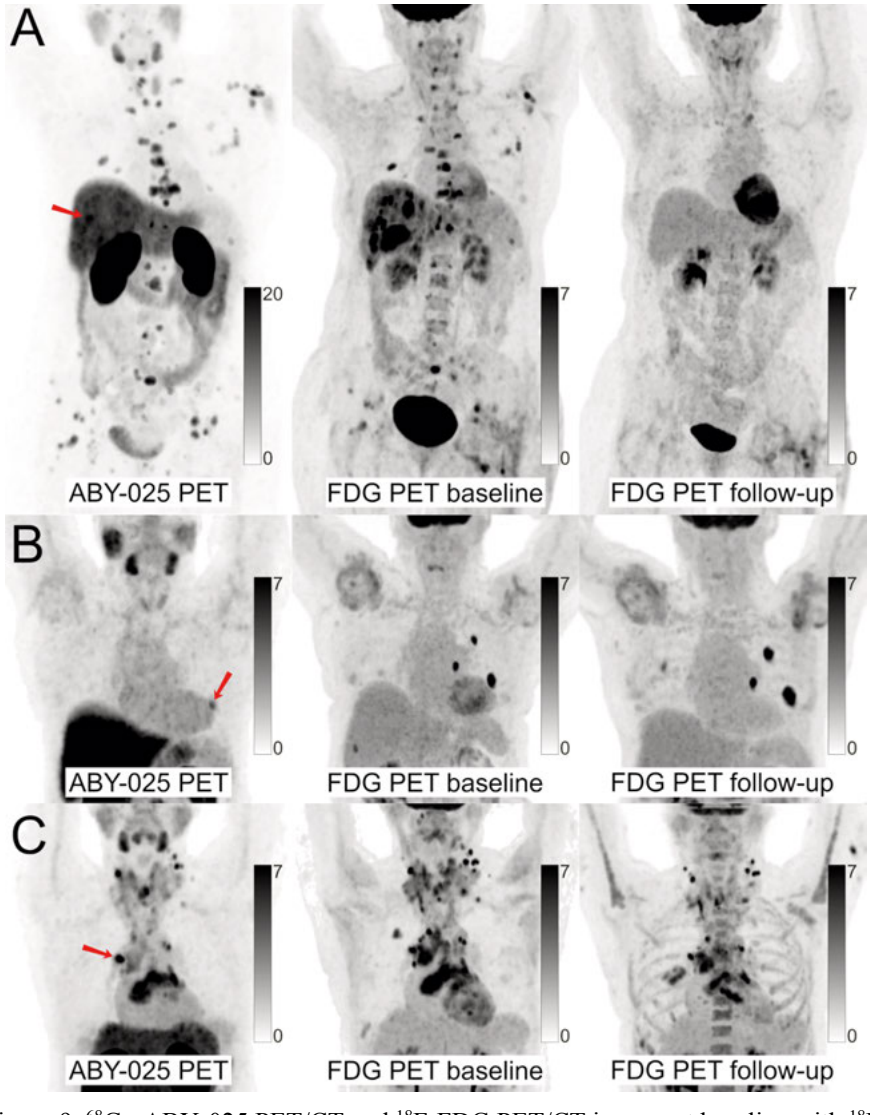

Figure 9. ^{68}Ga-ABY-025 PET/CT and ^{18}F-FDG PET/CT images at baseline with ^{18}F-FDG PET/CT follow-up after 2 cycles of treatment in biopsy-confirmed HER2-positive disease. (A) Patient with high ^{68}Ga-ABY-025 uptake (SUVmax, 21), previously received 3 lines of treatment. ^{18}F-FDG PET/CT follow-up showed complete metabolic response. (B) Patient with low uptake (SUVmax, 5.4), who previously received 3 lines of treatment. ^{18}F-FDG PET/CT follow-up showed disease progression (Δ-TLG, 168%) despite HER2-targeted treatment. (C) Patient with heterogeneous ^{68}Ga-ABY-025 uptake, who previously received 7 lines of treatment. ^{18}F-FDG PET/CT follow-up showed a heterogeneous response, with lesions higher in ^{68}Ga-ABY-025 uptake tending to have a better response. Arrows indicate biopsy sites.

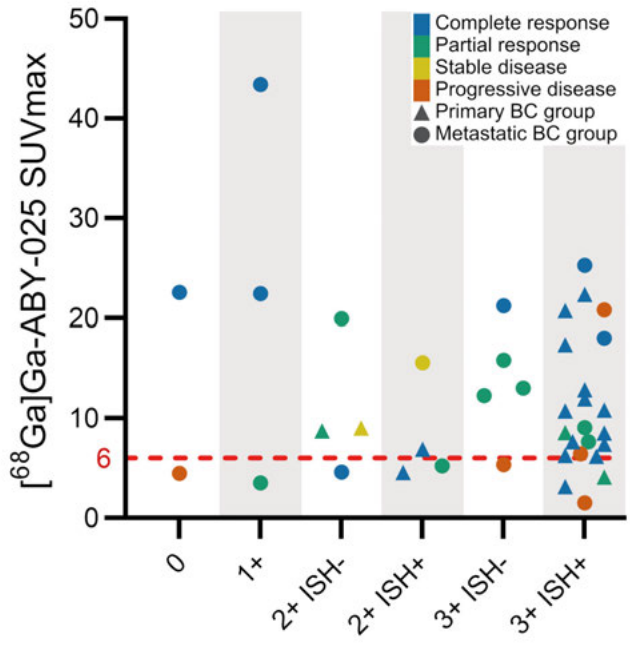

Figure 10. Clinical response in patients with breast cancer. The X-axis is HER2 status according to IHC and ISH from trial biopsies. The Y-axis corresponds to ^{68}Ga-ABY-025 PET uptake in biopsied lesions (n = 40). BC = breast cancer

We found that the metabolic response was associated with the number of previous treatments received. The more previous treatments the patients received, the worse the metabolic response measured (Figure 11). In a multivariable analysis, both the number of previous treatments and ^{68}Ga-ABY-025 PET SUVmax significantly correlated to the change in global Δ-TLG per patient. (p = 0.0004 and p = 0.018, respectively). Neither biopsy-derived HER2 status nor baseline TLG were found significant in this analysis (p = 0.09 and p = 0.17, respectively) (n = 39). On a lesional level, Δ-TLG association with ^{68}Ga-ABY-025 SUVmax, adjusted for the number of previous treatments, remained significant (R^2 = 0.30; n = 133; p = 0.0009). Although Δ-TLG was more prominent in PBC group (Average, -71%; 95% CI, -58% to -83%) compared to MBC group (Average, -27%; 95% CI, -16% to -38%; p < 0.0001), average ^{68}Ga-ABY-025 uptake did not differ significantly between PBC and MBC groups (SUVmax of 9.8 and 13.9 respectively; p = 0.10).

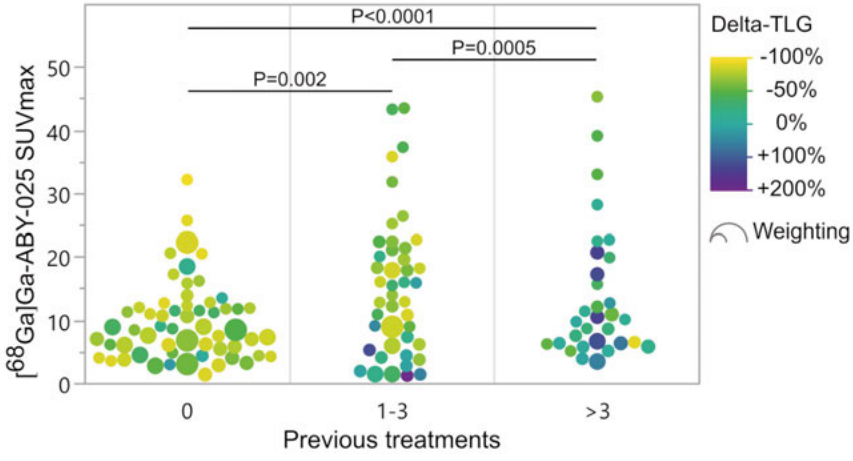

Figure 11. Number of previous treatments and their effect on response rate measured as Δ-TLG after 2 cycles of HER2-targeted treatment. One-way ANOVA showed significantly different response rates among 3 groups ($p < 0.0001$). Marker size reflects statistical weight of lesions per patient. P values represent Wilcoxon signed-rank test with regard to metabolic response among 3 groups.

Paper III

In paper III, we analyzed ^{68}Ga-ABY-025 PET and ^{18}F-FDG PET images in 50 patients (same patients in paper II + 10 additional patients included in the Affibody-3 clinical trial). We examined the role of ^{68}Ga-ABY-025 PET in proper staging and treatment planning, and the physiological and pathological uptake patterns in both treatment-naïve and recurrent breast cancer patients.

In this cohort, three patients had an additional disease spread into local axillary, supraclavicular, or internal thoracic lymph nodes that are only detectable with ^{68}Ga-ABY-025 PET (Figure 12). These findings helped in restaging in 2 out of 3 cases, both had invasive lobular carcinoma, which eventually led to adjustments in their treatment plan. All three patients responded to treatment with two of them achieving complete response early during treatment. The third patient had already liver metastasis detectable on both ^{68}Ga-ABY-025 PET and ^{18}F-FDG PET, thus continued with the same neoadjuvant treatment regimen. Unfortunately, she developed severe cardiac toxicity early during HER2-targeted treatment mandating discontinuation of HER2-targeted treatment, and her condition progressed shortly afterwards with brain metastasis that caused death.

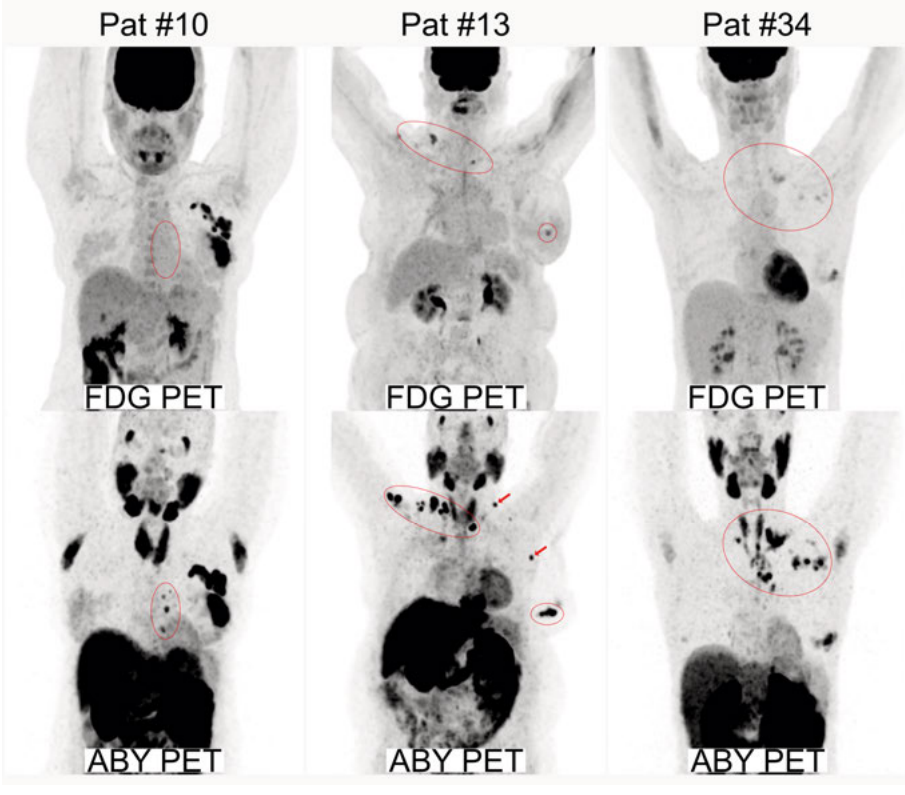

Figure 12. Patients with low metabolic activity in tumor lesions not detectable by ^{18}F-FDG PET, the same lesions were detectable using ^{68}Ga ABY-025 PET (patient #10 had ductal carcinoma, while patient #13 and patient #34 had lobular carcinoma).

Two patients in this cohort underwent the baseline scans shortly after getting COVID19 vaccine. Their ^{18}F-FDG PET showed an increased uptake in multiple lymph nodes ipsilateral to the injection site of the vaccine. The same lymph nodes did not have an increased uptake above normal background tissue on ^{68}Ga-AY-025 PET (Figure 13). Uptake in these vaccine-related lymph nodes disappeared in the follow-up ^{18}F-FDG PET.

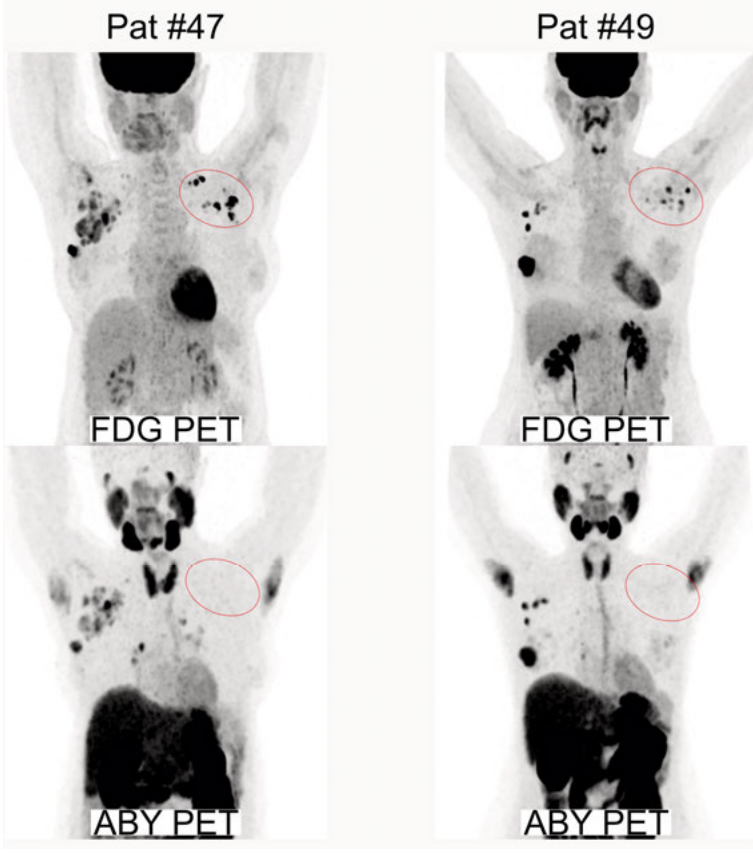

Figure 13. Two patients with non-malignant metabolic activity in lymph nodes on the ipsilateral side of COVID19 vaccine administration, visualized with FDG-PET (regions outlined in red). Vaccine-induced inflammation was not associated with increased ^{68}Ga-ABY-025 uptake.

^{68}Ga-ABY-025 uptake in the thyroid gland and axillary sweat glands was inversely correlated to the number of previous treatments (Figure 14). One patient in this cohort presented with a nodular uptake that is directly located above the thyroid gland. The subsequent investigation and biopsy results showed a benign pathology.

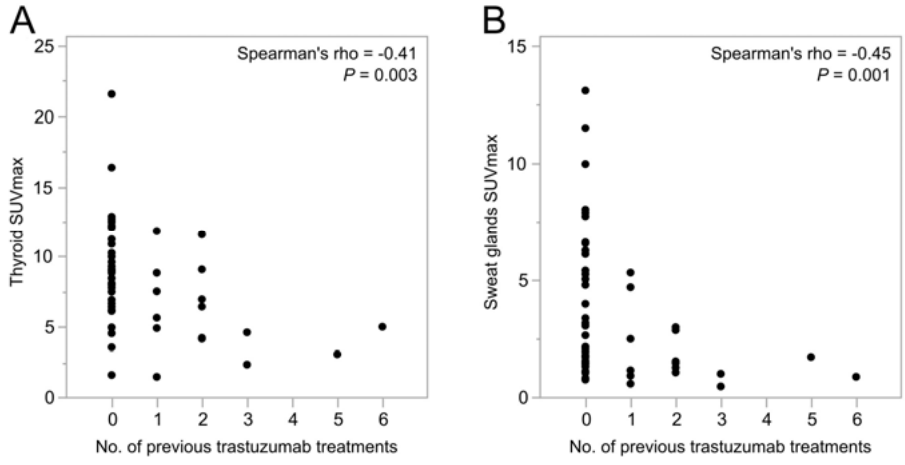

Figure 14. Spearman's correlations between the number of previous trastuzumab treatments received and ^{68}Ga-ABY-025 uptake in **A** thyroid gland and **B** axillary sweat glands in HER2-positive breast cancer patients (n = 50).

Paper IV

In this retrospective population-based study, we estimated the risk of developing BM in HER2-positive breast cancer and how it relates to other molecular subtypes. We also investigated the overall survival according to these subtypes, and the role of other patient-related and disease-related factors in both the risk of BM and survival.

Out of 663 patients, 134 had BM (Table 3). We found that the risk of developing BM 5 years after the first metastatic site was highest in hormonal receptor (HR) negative / HER2-positive (HR-/HER2+) subtype (41%; 95% CI 26 – 57%) followed by triple-negative subtype (39%; 95% CI 24 – 56%), HR+/HER2+ subtype (30%; 95% CI 19 – 44%), then HR+/HER2- subtype (14%; 95% CI 11 – 18%). The risk of developing BM in all patients was 19% (95% CI 16 – 23%).

Lobular disease (17.9% of cases) carried a higher risk for leptomeningeal involvement than ductal disease (72.2% of cases) (Hazard ratio (HzR), 2.85; 95% CI 1.74 – 4.67), while HzR for developing leptomeningeal disease was 0.19 in HER2-positive patients compared to HER2-negative patients (95% CI 0.06 – 0.58) ($p < 0.001$).

Table 3. Patient characteristics in paper IV.

All cohort	**HR+/HER2- (n=480)**	**HR+/HER2+ (n=64)**	**HR-/HER2+ (n=51)**	**Triple-negative (n=68)**	**Total (n=663)**
Mean age at MBC (± SD), y	**60 ± 13.9**	**58.4 ± 16.9**	**59.7 ± 12.8**	**58.2 ± 17.4**	**59.6 ± 14.5**
Histology					
Ductal	327 (68.1%)	58 (90.6%)	39 (76.5%)	55 (80.9%)	479 (72.2%)
Lobular	111 (23.1%)	3 (4.7%)	1 (2%)	4 (5.9%)	119 (17.9%)
Others	21 (4.4%)	0 (0%)	3 (5.9%)	5 (7.4%)	29 (4.4%)
Unknown	21 (4.4%)	3 (4.7%)	8 (15.7%)	4 (5.9%)	36 (5.4%)
De novo MBC	103 (21.5%)	18 (28.1%)	16 (31.4%)	12 (17.6%)	149 (22.5%)
Risk of CNS met	68 (14.2%)	22 (34.4%)	22 (43.1%)	22 (32.4%)	134 (20.2%)
Only CNS	***(n=68)***	***(n=22)***	***(n=22)***	***(n=22)***	***(n=134)***
De novo CNS at MBC	19 (27.9%)	7 (31.8%)	11 (50%)	9 (40.9%)	46 (34.3%)
Oligo met (1–**3)**	35 (51.5%)	12 (54.5%)	10 (45.5%)	13 (59.1%)	70 (52.2%)
Extra CNS met	62 (91.2%)	21 (95.5%)	19 (86.4%)	17 (77.3%)	119 (88.8%)
Meningeal met	30 (44.8%)	2 (9.5%)	1 (4.5%)	3 (13.6%)	36 (27.3%)
Radiotherapy	54 (79.4%)	19 (86.4%)	15 (68.2%)	18 (81.8%)	106 (79.1%)
Whole Brain	41 (60.3%)	15 (68.2%)	12 (54.5%)	12 (54.5%)	80 (59.7%)
Local	8 (11.8%)	2 (9.1%)	0 (0%)	1 (4.5%)	11 (8.2%)
Both WB + Local RT	5 (7.4%)	2 (9.1%)	3 (13.6%)	5 (22.7%)	15 (11.2%)
Surgery	3 (4.4%)	2 (9.1%)	2 (9.1%)	3 (13.6%)	10 (7.5%)
Gamma knife	0 (0%)	0 (0%)	0 (0%)	1 (4.5%)	1 (0.7%)
Systemic Rx					
Chemotherapy	49 (72.1%)	19 (86.4%)	15 (68.2%)	17 (77.3%)	100 (74.6%)
Antibodies	7 (10.3%)	17 (77.3%)	13 (59.1%)	2 (9.1%)	39 (29.1%)
Endocrine	27 (39.7%)	8 (36.4%)	0 (0%)	1 (4.5%)	36 (26.9%)
Outcome CNS					
Response Rate	25 (36.8%)	14 (63.6%)	13 (59.1%)	8 (36.4%)	60 (44.8%)
Complete Response	7 (10.3%)	6 (27.3%)	6 (27.3%)	5 (22.7%)	24 (17.9%)
Median OS (months)	7.7	26.2	11.2	3.5	8.8
Deaths caused by BM	41 (60.3%)	11 (50%)	16 (72.7%)	10 (45.5%)	78 (58.2%)

MBC = metastatic breast cancer, CNS = central nervous system, WB = whole brain, RT = radiotherapy, OS = overall survival, BM = brain metastasis

Among the molecular subtypes, HR+/HER2+ had the longest survival after the first metastatic site at 63 months (95% CI 42 – 72), followed by HR+/HER2-with 43 months (95% CI 37 – 51). HR-/HER2+ and triple-negative subtypes had 36 months (95% CI 19 – 53) and 16 months (95% CI 8 – 23), respectively ($p = 0.002$) (Figure 15 B).

HR+/HER2+ subtype also had the longest survival after developing BM (26.2 months; 95% CI 14 – 43), followed by HR-/HER2+ subtype (11.2 months; 95% CI 3 – 29), HR+/HER2- subtype (7.7 months; 95% CI 4 – 14), and lastly triple-negative subtype with the worst survival of only 3.5 months (95% CI 2 – 9; $p = 0.04$) (Figure 15 A). Time to develop BM was relatively short and comparable in both HER2+ subtypes as well as triple-negative disease but was significantly longer in HR+/HER2- subtype (figure 15 C and D).

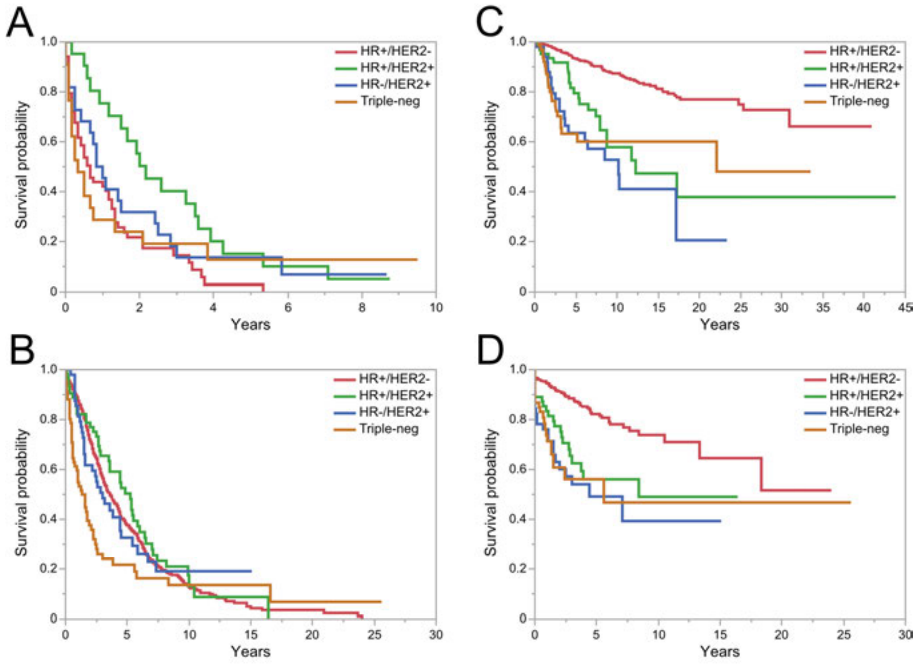

Figure 15. **A** Overall survival after developing BM in breast cancer patients based on their molecular subtypes [HR+/HER2- (n = 68), HR+/HER2+ (n = 22), HR+/HER2+ (n = 22), HR-/HER2- (n = 22)], (total n=134, $p = 0.053$), **B** Overall survival after first metastatic site in breast cancer based on their molecular subtypes [HR+/HER2- (n = 480), HR+/HER2+ (n = 64), HR+/HER2+ (n = 51), HR-/HER2- (n = 68)], (total n=663, p<0.0001), **C** time to develop BM from initial diagnosis [HR+/HER2- (n = 480), HR+/HER2+ (n = 64), HR+/HER2+ (n = 51), HR-/HER2- (n = 68)], (total n=663, $p < 0.0001$), and **D** time to BM from first metastasis [HR+/HER2- (n = 480), HR+/HER2+ (n = 64), HR+/HER2+ (n = 51), HR-/HER2- (n = 68)], (total n=663, $p = 0.0004$).

We found that patients with breast cancer brain metastasis (BCBM) without extra CNS involvement as well as patients with BM as first metastatic site (de novo BM) had a favorable survival. Patients with BM without extra CNS involvement had a longer survival of 16 months (95% CI 5 – 46; $n = 15$), while patients with extra CNS involvement had a median survival of only 9 months (95% CI 6 – 15) ($n = 119$; $p = 0.18$). Overall survival after BM was significantly longer (18 months, 95% CI 12 – 36) in patients with BM as the first metastatic site (de novo BM) compared to the recurrent disease (6 months, 95% CI 3 – 9; $p = 0.001$).

We investigated the influence of various treatment modalities on overall survival as well. In patients who underwent surgical removal of BM, median overall survival was much longer (46 months, 95% CI 9 – 104) compared to those who did not undergo surgical removal of BM (8 months, 95% CI 6 – 13; $p = 0.013$). Patients with BM who received radiotherapy had a significantly

higher overall survival (13 months, 95% CI 8 – 16) than BM patients who did not receive radiotherapy (2 months, 95% CI 1 – 10) ($p = 0.03$). Radiotherapy target and protocol influenced the overall survival as well. Patients with BCBM who received adjuvant radiotherapy to the tumor and/or stereotactic radiosurgery had the best overall survival of 41 months (95% CI 8 – 89). Patients who had whole brain palliative radiotherapy with or without a boost to the tumor had an overall survival of 18 months (95% CI 3 – 42) and 10 months (95% CI 7 – 14), respectively. While for patients with BM who did not receive any radiotherapy, the overall survival was only 2 months (95% CI 1 – 10) ($p = 0.006$).

Survival was significantly longer for patients with 1 to 3 brain lesions (12 months, 95% CI 7 – 18) compared with patients with more than 3 brain lesions (8 months, 95% CI 4 – 14; $p = 0.02$) (Figure 16).

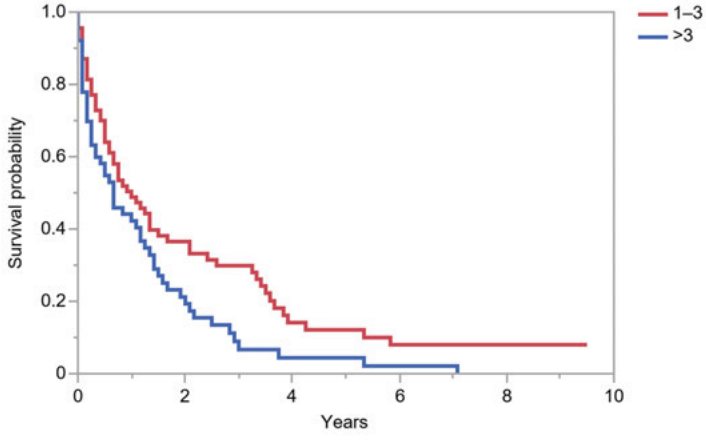

Figure 16. Overall survival in patients with BM based on the number of metastatic lesions, median survival was 8 months (95% CI 4 – 14) in patients with > 3 lesions (n = 64), while 12 months (95% CI 7 – 18) in patients with 1 to 3 lesions (n = 70), ($p = 0.016$).

For systemic HER2-targeted therapy, BCBM patients with HER2-positive disease who received HER2-targeted treatment had a longer survival (26 months, 95% CI 14 – 36) than those who did not receive HER2-targeted therapy (7,5 months, 95% CI 0 – 18) and those with HER2-negative disease (6 months, 95% CI 4 – 12) ($p = 0.01$) (Figure 17).

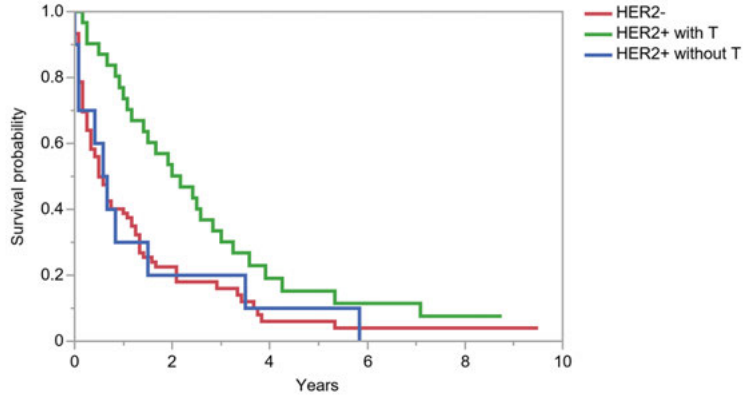

Figure 17. Overall survival of BCBM, comparing HER2+ cases with trastuzumab, HER2+ without trastuzumab, and HER2- cases. Median survival time for HER2+ patients with trastuzumab treatment was 26 months (95% CI 14 – 36), while for HER2+ patients without trastuzumab treatment and HER2- patients was 7.5 months (95% CI 0 – 18) and 6 months (95% CI 4 – 12), respectively ($p = 0.01$).

In a univariate analysis, factors such as age < 55 years, HER2 positivity, HR negativity or triple-negative disease, Ki-67 ≥ 30%, pathological grade (Elston) 3, and de novo MBC carried a higher risk for developing BM (Figure 18).

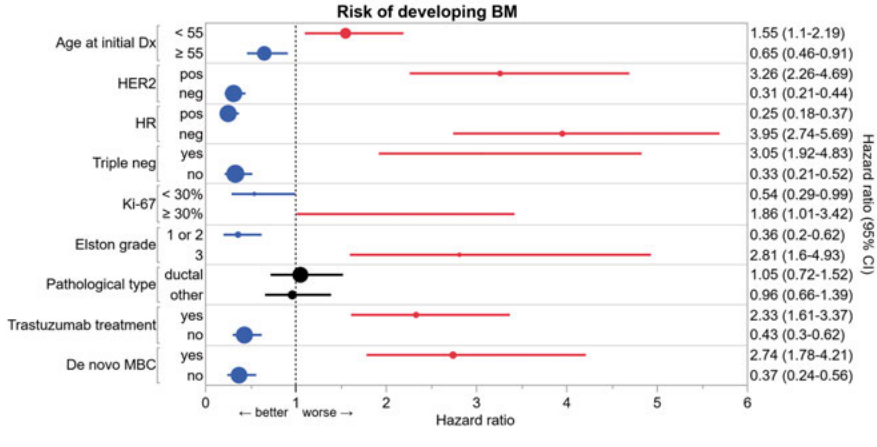

Figure 18. Forest plot showing hazard ratios of several factors to develop BM from primary diagnosis in metastatic breast cancer patients treated in Uppsala County between 2009 and 2022 calculated via univariate analysis. BM = brain metastasis

Factors that increased mortality after BM were HER2 negativity, lack of systemic therapy including chemotherapy and targeted therapy, no radiotherapy, and multiple metastatic brain lesions (>3) (Figure 19).

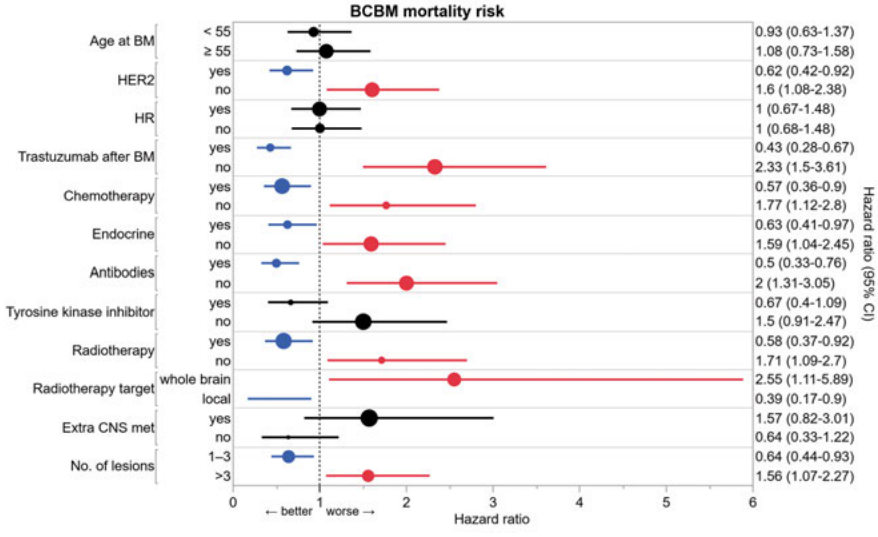

Figure 19. Forest plot showing hazard ratios for BCBM mortality risk of several factors in metastatic breast cancer patients treated in Uppsala County between 2009 and 2022 calculated via univariate analysis. BCBM = breast cancer brain metastasis, HR = hormonal receptor

Discussion

Current guidelines to determine HER2 status is limited to biopsy specimens which within the heterogeneous HER2 expression in breast cancer is suboptimal. This makes introducing an imaging technique to quantify HER2 status in the whole body advantageous. ABY-025 molecule showed high affinity and selectivity towards HER2 receptors. Using ^{68}Ga-ABY-025 PET, it was possible to quantify HER2 status and to predict treatment outcome in patients with MBC.

The results from paper I showed that the irreversible two-tissue compartment model fitted the kinetics of ^{68}Ga-ABY-025 best, which came in agreement with the preclinical findings that showed almost irreversible kinetic properties of Affibody molecules in HER2 expressing tumor-cell lines.[72,73] However, we found a small reversible component detectable in liver lesions. We suspect that it is due to the spill-in effect from the surrounding normal liver uptake.

Based on our findings, VOI-based kinetic modeling and voxel-based parametric images were identical and can be used interchangeably. The basis function implementation of the 2TC-3k model provided K_1 and K_i parametric images. SUV values strongly correlated to both 2TC K_i ($R^2 = 0.87$, $n = 9$) values and Patlak K_i ($R^2 = 0.95$, $n = 9$) values, which means that SUVs mostly reflect the actual binding of the tracer in tumor lesions, thus supporting its use in HER2 imaging and quantification.

We found that the ^{68}Ga-ABY-025 uptake in normal liver was relatively high. The liver is where peptides such as the Affibody molecule is metabolized. However, a low to moderate degree of HER2 expression in normal liver has also been previously reported.[74,75] This might explain the specific uptake still found in normal liver detectable in parametric K_i images. The majority of lesions in paper I were in the liver. Patlak graphical analysis assumes irreversible binding which led in our case to the underestimation of K_i values in normal liver. This might explain the higher tumor-to-liver ratios we found with Patlak parametric images, which in certain clinical cases could make it easier to detect small liver lesions.

Parametric images should be preserved to certain cases as they require a 45-minute dynamic PET scan that puts a logistic burden on both the patient and healthcare providers. In addition, parametric images require special programs to create that might not be widely available.

In paper II, discordant results between ^{68}Ga-ABY-025 PET uptake and biopsy-based HER2 status were found in 12 out of 40 patients with no significant correlation found between the two. However, we were able to accurately predict the metabolic response to treatment using results from ^{68}Ga-ABY-025 PET. This was not possible with biopsy-based results where no significant predictive value was found, suggesting that biopsy-based HER2 status did not necessarily reflect the receptors bioavailability. Breast cancer is known for its heterogeneity and discordances between the primary tumor and metastases, as well as intratumoral heterogeneity have been reported.[40,76] Low ^{68}Ga-ABY-025 uptake despite HER2-positive biopsy could mean that HER2-targeted antibodies, such as trastuzumab, would not have access to the receptors, leading to poor response. On the other hand, high ^{68}Ga-ABY-025 uptake despite HER2-negative biopsy could be the result of tumor heterogeneity or, in some cases, failed sampling. The latter is particularly more common in liver and skeletal lesions where biopsies are relatively difficult to obtain compared to other sites.

We found that the response to treatment was inversely correlated to the number of previous treatments received by patients prior to this study. Hence, all treatment-naïve patients responded to treatment but to a variable degree irrespective of ^{68}Ga-ABY-025 uptake. This variation in response could reflect some degree of initial resistance in some of these patients. ^{68}Ga-ABY-025 uptake together with the number of previous treatments could explain 30% (R^2 = 0.30) of the metabolic response. A part of the remaining 70% could be attributed to other factors, such as clonal variation of primary or acquired drug resistance to HER2-targeted therapy resulting from exposure to previous treatments.[31,45]

Using a predefined cutoff SUVmax of 6.0, it was possible to predict positive metabolic response in lymph nodes and soft tissue lesions. The same cutoff could not be applied to other tissues such as skeletal lesions, where a higher cutoff SUVmax value of 16.2 was required. Skeletal metastases are usually associated with a later stage of the disease and are generally found in patients who had more likely received multiple previous treatments, which in turn are linked to drug resistance. Metabolic flare effect that is usually associated with the inflammatory response in bone lesions could also affect adequate estimation of metabolic response using ^{18}F-FDG PET.[77]

^{68}Ga-ABY-025 PET provided a non-invasive tool allowing for whole-body evaluation of HER2-receptor expression. A tool that is particularly useful in situations where biopsies are difficult to obtain or when disease heterogeneity is suspected. ^{68}Ga-ABY-025 PET might provide additional information that helps identify patients most likely to benefit from HER2-targeted treatment thus avoiding unnecessary side effects and ensuring timely treatment planning. This has become increasingly relevant since the introduction of new lines of treatment to target HER2-low expression.[78,79]

As HER2-targeted imaging becomes more widely used in clinical settings, paper III offers an overview of diagnostic prospects and possible difficulties associated with HER2 PET that might be clinically relevant. We found that in some cases, ^{68}Ga-ABY-025 PET provided additional information that led to restaging and adjustment of the treatment regimen, thereby guiding the treatment decision and sparing patients from unnecessary side effects. The advantages that receptor-targeted imaging has over ^{18}F-FDG PET have been reported with other tracers as well, such as ^{18}F-FES in ER-positive breast cancer. More lesions are detected using ^{18}F-FES PET compared to ^{18}F-FDG PET.[80] In paper III, two of the three patients with positive ^{68}Ga-ABY-025 PET findings undetectable by 18F-FDG PET had lobular disease. Lobular carcinomas are historically known to have a slow growth rate relative to other types and a growth pattern that makes them difficult to detect in mammography. These characteristics could explain the low metabolic uptake in ^{18}F-FDG PET.[81]

Treatment decision of locally advanced breast cancer depends on accurate staging, which in turn depends on the proper detection of the involved lymph nodes. ^{68}Ga-ABY-025 PET findings helped guide definitive treatment in these patients leading to a better prognosis. These benefits however are limited to HER2-expressing tumors.

Non-malignant inflammatory or immunologically induced reactions to vaccinations are a source of false-positive findings in tracers that measure the metabolic activity. Since the introduction of COVID19 vaccine, several studies have reported increased uptake with ^{18}F-FDG and ^{11}C-choline PET in cervical, axillary, and clavicular lymph nodes ipsilateral to the intramuscular vaccine injection site in the deltoid muscle.[82–86] while more specific tracers, such as ^{68}Ga-FAPI PET, provide a tool to minimize false positive findings.[87]

So far, ^{68}Ga-ABY-025 uptake appears to be less impacted by benign inflammatory processes allowing to distinguish malignant from non-malignant lesions. Similar findings were reported previously with PSMA-targeted tracers in prostate cancer and somatostatin-receptor-targeted tracers in neuroendocrine tumors.[88–91]

We found that uptake in the thyroid gland was higher than background uptake and is more prominent in the treatment-naïve group of patients. The uptake in the thyroid gland was inversely proportional to the number of previous treatments received. Exposure to multiple lines of chemotherapy has been associated with signs and symptoms of reduced thyroid function such as fatigue, lethargy, amenorrhea, and weight gain.[92–94] Our findings suggest that there is a link between ^{68}Ga-ABY-025 uptake in the thyroid gland and its function which we think is worth considering in future studies. The increased uptake in axillary sweat glands might not be of great clinical significance but it is worth noting for imaging specialists new to HER2-imaging. Previous research has reported normal expression of HER2 receptors in apocrine sweat glands.[95]

In paper IV, the majority of patients with MBC were of HR+/HER2- subtype (72%), 17% had HER2-positive subtype, while 10% of patients had triple-negative disease. This came in agreement with similar studies.[96,97] The median overall survival of BCBM was 8.8 months, which is slightly longer but in line with results reported by other studies.[98–101] HER2-positive molecular subtypes carried the highest risk of developing BM, followed closely by triple-negative subtype. These findings are comparable to what have been reported by other studies.[102,103] HER2 expression is linked with aggressiveness and an increased tendency to metastasize.[104] Despite the comparable risk of developing BM between HER2+ and triple-negative subtypes, HER2+ subtypes had a significantly longer overall survival. This improved outcome is most likely a product of more personalized treatment with HER2-targeted antibodies like trastuzumab or antibody-drug conjugates like trastuzumab emtansine. Despite the impermeability of BBB to antibodies, studies have shown that metastatic brain lesions over 0.5 cm affect the integrity of the BBB, rendering it permeable to large molecules including anti-cancer regimens such as HER2-targeted antibodies.[105–107] Park et al. reported that trastuzumab treatment given to HER2-positive breast cancer patients with BM is associated with improved survival.[108] In our study, trastuzumab treatment after BCBM significantly improved survival outcome, even when compared with HR+/HER2-. The latter showed a lower overall survival of 7.7 months. HR+/HER2- subtype tends to develop BM at a later stage of the disease which might explain the shorter survival after BM.

Similar to antibodies, systemic chemotherapy had a positive effect on disease outcome and improved survival almost two-fold. The benefit of systemic chemotherapy on overall survival came as expected and has been reported previously.[99,106] Local treatment in the form of surgery and radiotherapy showed an impact on survival as well. Fewer metastatic brain lesions were also associated with longer overall survival. However, the decision to give radiotherapy with a curative intent or palliative whole-brain RT is based on the disease status including the number of metastatic lesions in the brain. In our study, patients with 3 or fewer BM lesions had significantly longer survival (12 months) than patients with more than 3 lesions (8 months; $p = 0.02$). Additionally, the decision to give or withhold treatment relies on the general condition of the patient. In patients with poor general conditions that are not expected to survive longer, treatment might be withheld. This could introduce a selection bias, thus our findings should be cautiously interpreted.

Our findings showed that patients with de novo BM and the lack of metastases outside CNS had a favorable survival. These patients usually presented with less cancer-related morbidity that contributed to longer survivability. Lobular carcinoma was associated with an increased risk of leptomeningeal disease (HzR = 2.85). Leptomeningeal disease is a serious complication presented with neurological symptoms that affect patients' quality of life and carry a poor prognosis.[109,110]

Concluding remarks and future perspectives

In general, this thesis showed that imaging HER2 receptors in breast cancer patients was possible using the novel tracer ^{68}Ga-ABY-025, allowing for whole-body HER2 receptor quantification with the non-invasive PET imaging that helped predict treatment outcome. More specific conclusions made from this work are:

^{68}Ga-ABY-025 kinetics were better explained using the irreversible two-tissue compartment model, tumor K_i values showed a strong correlation with the SUV values both 2 and 4 h post-injection, supporting its use to reflect actual ^{68}Ga-ABY-025 binding to HER2 receptor. Parametric K_i images facilitated the visualization of small lesions in organs with known physiological uptake such as the liver.

Molecular imaging using ^{68}Ga-ABY-025 PET predicted treatment outcome in breast cancer patients receiving HER2-targeting therapy with higher specificity compared to biopsy results. Patients who had received several prior treatments had a lower response rate to therapy with higher ^{68}Ga-ABY-025 uptake was necessary to achieve the desired metabolic response.

^{68}Ga-ABY-025 PET might serve as a useful tool for accurate staging and a supplement to ^{18}F-FDG PET, particularly in cases of HER2-expressing breast cancer with lower metabolic activity, in patients with concurrent inflammatory and malignant lesions, and in small lesions that are difficult to detect with other imaging modalities. Cancer treatments influence the uptake of ^{68}Ga-ABY-025 in some healthy tissues such as the thyroid and sweat glands.

Among breast cancer molecular subtypes, patients with HER2-positive breast cancer had the longest survival following BM diagnosis, but they also have a higher risk of developing BM. Systemic treatment, including antibodies targeting HER2 receptors, has a positive effect on the overall prognosis of BCBM. BM was considered the direct cause of death in 70% of diseased BCBM patients.

Future research should be tailored towards a more clinically oriented questions. Other researchers already started working on imaging patients with HER2-low expression, which is highly relevant especially with the recent advancements in HER2-targeted treatments. Another aspect is the use of ABY-025 analogs labeled with beta-emitting isotopes as radionuclide therapy for breast cancer, which is still in the preclinical phase but has shown promising results.[111]

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References

1 Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A *et al.* Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin* **71**, 209–249 (2021).

2 Official Statistics of Sweden, Health and Medical Care, National Board of Health and Welfare Art . no: 2022-12-8308. (2022)https://www.socialstyrelsen.se/globalassets/sharepoint-dokument/artikelkatalog/statistik/2022-12-8308.pdf (accessed 22 Aug2023).

3 Hulka BS, Stark AT. Breast cancer: cause and prevention. *The Lancet* **346**, 883–887 (1995).

4 Kamińska M, Ciszewski T, Łopacka-Szatan K, Miotła P, Starosławska E. Breast cancer risk factors. *Prz Menopauzalny* **14**, 196–202 (2015).

5 Lima SM, Kehm RD, Terry MB. Global breast cancer incidence and mortality trends by region, age-groups, and fertility patterns. *EClinicalMedicine* **38**, 100985 (2021).

6 DeSantis CE, Bray F, Ferlay J, Lortet-Tieulent J, Anderson BO, Jemal A. International Variation in Female Breast Cancer Incidence and Mortality Rates. *Cancer Epidemiol Biomarkers Prev* **24**, 1495–1506 (2015).

7 Elston CW, Ellis IO. Pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: experience from a large study with long-term follow-up. *Histopathology* **19**, 403–410 (1991).

8 Ellis IO, Galea M, Broughton N, Locker A, Blamey RW, Elston CW. Pathological prognostic factors in breast cancer. II. Histological type. Relationship with survival in a large study with long-term follow-up. *Histopathology* **20**, 479–489 (1992).

9 Rakha EA, Green AR. Molecular classification of breast cancer: what the pathologist needs to know. *Pathology* **49**, 111–119 (2017).

10 Perou CM, Sørlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA *et al.* Molecular portraits of human breast tumours. *Nature* **406**, 747–752 (2000).

11 Citri A, Yarden Y. EGF-ERBB signalling: towards the systems level. *Nat Rev Mol Cell Biol* **7**, 505–516 (2006).

12 Press MF, Cordon-Cardo C, Slamon DJ. Expression of the HER-2/neu proto-oncogene in normal human adult and fetal tissues. *Oncogene* **5**, 953–962 (1990).

13 Ross JS, Fletcher JA, Bloom KJ, Linette GP, Stec J, Clark E *et al.* HER-2/neu testing in breast cancer. *Am J Clin Pathol* **120 Suppl**, S53-71 (2003).

14 Furrer D, Paquet C, Jacob S, Diorio C, Furrer D, Paquet C *et al.* The Human Epidermal Growth Factor Receptor 2 (HER2) as a Prognostic and Predictive Biomarker: Molecular Insights into HER2 Activation and Diagnostic Implications. In: *Cancer Prognosis*. IntechOpen, 2018 doi:10.5772/intechopen.78271.

15 Diermeier S, Horváth G, Knuechel-Clarke R, Hofstaedter F, Szöllosi J, Brockhoff G. Epidermal growth factor receptor coexpression modulates susceptibility to Herceptin in HER2/neu overexpressing breast cancer cells via specific erbB-receptor interaction and activation. *Exp Cell Res* **304**, 604–619 (2005).
16 Slamon DJ, Godolphin W, Jones LA, Holt JA, Wong SG, Keith DE *et al.* Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. *Science* **244**, 707–712 (1989).
17 Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science* **235**, 177–182 (1987).
18 Carlsson J, Nordgren H, Sjöström J, Wester K, Villman K, Bengtsson NO *et al.* HER2 expression in breast cancer primary tumours and corresponding metastases. Original data and literature review. *Br J Cancer* **90**, 2344–2348 (2004).
19 Dahabreh IJ, Linardou H, Siannis F, Fountzilas G, Murray S. Trastuzumab in the adjuvant treatment of early-stage breast cancer: a systematic review and meta-analysis of randomized controlled trials. *Oncologist* **13**, 620–630 (2008).
20 De Laurentiis M, Cancello G, D'Agostino D, Giuliano M, Giordano A, Montagna E *et al.* Taxane-based combinations as adjuvant chemotherapy of early breast cancer: a meta-analysis of randomized trials. *J Clin Oncol* **26**, 44–53 (2008).
21 Fujii T, Le Du F, Xiao L, Kogawa T, Barcenas CH, Alvarez RH *et al.* Effectiveness of an Adjuvant Chemotherapy Regimen for Early-Stage Breast Cancer: A Systematic Review and Network Meta-analysis. *JAMA Oncol* **1**, 1311–1318 (2015).
22 Zardavas D, Piccart M. Neoadjuvant therapy for breast cancer. *Annu Rev Med* **66**, 31–48 (2015).
23 Sirohi B, Arnedos M, Popat S, Ashley S, Nerurkar A, Walsh G *et al.* Platinum-based chemotherapy in triple-negative breast cancer. *Ann Oncol* **19**, 1847–1852 (2008).
24 Gianni L, Pienkowski T, Im Y-H, Tseng L-M, Liu M-C, Lluch A *et al.* 5-year analysis of neoadjuvant pertuzumab and trastuzumab in patients with locally advanced, inflammatory, or early-stage HER2-positive breast cancer (NeoSphere): a multicentre, open-label, phase 2 randomised trial. *Lancet Oncol* **17**, 791–800 (2016).
25 Nanda R, Liu MC, Yau C, Shatsky R, Pusztai L, Wallace A *et al.* Effect of Pembrolizumab Plus Neoadjuvant Chemotherapy on Pathologic Complete Response in Women With Early-Stage Breast Cancer: An Analysis of the Ongoing Phase 2 Adaptively Randomized I-SPY2 Trial. *JAMA Oncol* **6**, 676–684 (2020).
26 Early Breast Cancer Trialists' Collaborative Group (EBCTCG). Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials. *Lancet* **365**, 1687–1717 (2005).
27 Altundag K, Ibrahim NK. Aromatase inhibitors in breast cancer: an overview. *Oncologist* **11**, 553–562 (2006).
28 Taskar KS, Rudraraju V, Mittapalli RK, Samala R, Thorsheim HR, Lockman J *et al.* Lapatinib distribution in HER2 overexpressing experimental brain metastases of breast cancer. *Pharm Res* **29**, 770–781 (2012).
29 Cameron D, Casey M, Press M, Lindquist D, Pienkowski T, Romieu CG *et al.* A phase III randomized comparison of lapatinib plus capecitabine versus capecitabine alone in women with advanced breast cancer that has progressed on

trastuzumab: updated efficacy and biomarker analyses. *Breast Cancer Res Treat* **112**, 533–543 (2008).

30 Lin NU, Borges V, Anders C, Murthy RK, Paplomata E, Hamilton E *et al.* Intracranial Efficacy and Survival With Tucatinib Plus Trastuzumab and Capecitabine for Previously Treated HER2-Positive Breast Cancer With Brain Metastases in the HER2CLIMB Trial. *J Clin Oncol* **38**, 2610–2619 (2020).

31 Vogel CL, Cobleigh MA, Tripathy D, Gutheil JC, Harris LN, Fehrenbacher L *et al.* Efficacy and safety of trastuzumab as a single agent in first-line treatment of HER2-overexpressing metastatic breast cancer. *J Clin Oncol* **20**, 719–726 (2002).

32 Cameron D, Piccart-Gebhart MJ, Gelber RD, Procter M, Goldhirsch A, de Azambuja E *et al.* 11 years' follow-up of trastuzumab after adjuvant chemotherapy in HER2-positive early breast cancer: final analysis of the HERceptin Adjuvant (HERA) trial. *Lancet* **389**, 1195–1205 (2017).

33 Cho H-S, Mason K, Ramyar KX, Stanley AM, Gabelli SB, Denney DW *et al.* Structure of the extracellular region of HER2 alone and in complex with the Herceptin Fab. *Nature* **421**, 756–760 (2003).

34 Pegram MD, Konecny G, Slamon DJ. The molecular and cellular biology of HER2/neu gene amplification/overexpression and the clinical development of herceptin (trastuzumab) therapy for breast cancer. *Cancer Treat Res* **103**, 57–75 (2000).

35 McKeage K, Perry CM. Trastuzumab: a review of its use in the treatment of metastatic breast cancer overexpressing HER2. *Drugs* **62**, 209–243 (2002).

36 Gianni L, Pienkowski T, Im Y-H, Roman L, Tseng L-M, Liu M-C *et al.* Efficacy and safety of neoadjuvant pertuzumab and trastuzumab in women with locally advanced, inflammatory, or early HER2-positive breast cancer (NeoSphere): a randomised multicentre, open-label, phase 2 trial. *Lancet Oncol* **13**, 25–32 (2012).

37 Swain SM, Baselga J, Kim S-B, Ro J, Semiglazov V, Campone M *et al.* Pertuzumab, trastuzumab, and docetaxel in HER2-positive metastatic breast cancer. *N Engl J Med* **372**, 724–734 (2015).

38 Wolff AC, Hammond MEH, Schwartz JN, Hagerty KL, Allred DC, Cote RJ *et al.* American Society of Clinical Oncology/College of American Pathologists Guideline Recommendations for Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer. *JCO* **25**, 118–145 (2006).

39 Wolff AC, Hammond MEH, Allison KH, Harvey BE, Mangu PB, Bartlett JMS *et al.* Human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline focused update. *Arch Pathol Lab Med* **142**, 1364–1382 (2018).

40 Cottu PH, Asselah J, Lae M, Pierga J-Y, Diéras V, Mignot L *et al.* Intratumoral heterogeneity of HER2/neu expression and its consequences for the management of advanced breast cancer. *Ann Oncol* **19**, 595–597 (2008).

41 Hou Y, Nitta H, Li Z. HER2 Gene Protein Assay Is Useful to Determine HER2 Status and Evaluate HER2 Heterogeneity in HER2 Equivocal Breast Cancer. *Am J Clin Pathol* **147**, 89–95 (2017).

42 Rimawi MF, De Angelis C, Schiff R. Resistance to Anti-HER2 Therapies in Breast Cancer. *American Society of Clinical Oncology Educational Book* , e157–e164 (2015).

43 Bartsch R, Wenzel C, Steger GG. Trastuzumab in the management of early and advanced stage breast cancer. *Biologics* **1**, 19–31 (2007).

44 Yeo B, Kotsori K, Mohammed K, Walsh G, Smith IE. Long-term outcome of HER2 positive metastatic breast cancer patients treated with first-line trastuzumab. *Breast* **24**, 751–757 (2015).
45 Pohlmann PR, Mayer IA, Mernaugh R. Resistance to trastuzumab in breast cancer. *Clin Cancer Res* **15**, 7479–7491 (2009).
46 Scaltriti M, Rojo F, Ocaña A, Anido J, Guzman M, Cortes J *et al.* Expression of p95HER2, a truncated form of the HER2 receptor, and response to anti-HER2 therapies in breast cancer. *J Natl Cancer Inst* **99**, 628–638 (2007).
47 Berns K, Horlings HM, Hennessy BT, Madiredjo M, Hijmans EM, Beelen K *et al.* A functional genetic approach identifies the PI3K pathway as a major determinant of trastuzumab resistance in breast cancer. *Cancer Cell* **12**, 395–402 (2007).
48 Serra V, Markman B, Scaltriti M, Eichhorn PJA, Valero V, Guzman M *et al.* NVP-BEZ235, a dual PI3K/mTOR inhibitor, prevents PI3K signaling and inhibits the growth of cancer cells with activating PI3K mutations. *Cancer Res* **68**, 8022–8030 (2008).
49 Lu Y, Zi X, Zhao Y, Mascarenhas D, Pollak M. Insulin-like growth factor-I receptor signaling and resistance to trastuzumab (Herceptin). *J Natl Cancer Inst* **93**, 1852–1857 (2001).
50 von Minckwitz G, Huang C-S, Mano MS, Loibl S, Mamounas EP, Untch M *et al.* Trastuzumab Emtansine for Residual Invasive HER2-Positive Breast Cancer. *N Engl J Med* **380**, 617–628 (2019).
51 Baselga J, Cortés J, Kim S-B, Im S-A, Hegg R, Im Y-H *et al.* Pertuzumab plus trastuzumab plus docetaxel for metastatic breast cancer. *N Engl J Med* **366**, 109–119 (2012).
52 Johnston SRD, Hegg R, Im S-A, Park IH, Burdaeva O, Kurteva G *et al.* Phase III, Randomized Study of Dual Human Epidermal Growth Factor Receptor 2 (HER2) Blockade With Lapatinib Plus Trastuzumab in Combination With an Aromatase Inhibitor in Postmenopausal Women With HER2-Positive, Hormone Receptor-Positive Metastatic Breast Cancer: Updated Results of ALTERNATIVE. *J Clin Oncol* **39**, 79–89 (2021).
53 Alcantara D, Leal MP, García-Bocanegra I, García-Martín ML. Molecular imaging of breast cancer: present and future directions. *Front Chem* **2**, 112 (2014).
54 Avril S, Muzic RF, Plecha D, Traughber BJ, Vinayak S, Avril N. ^{18}F-FDG PET/CT for Monitoring of Treatment Response in Breast Cancer. *J Nucl Med* **57 Suppl 1**, 34S–9S (2016).
55 Groheux D, Cochet A, Humbert O, Alberini J-L, Hindié E, Mankoff D. ^{18}F-FDG PET/CT for Staging and Restaging of Breast Cancer. *J Nucl Med* **57 Suppl 1**, 17S-26S (2016).
56 Culverwell AD, Scarsbrook AF, Chowdhury FU. False-positive uptake on 2-[^{18}F]-fluoro-2-deoxy-D-glucose (FDG) positron-emission tomography/computed tomography (PET/CT) in oncological imaging. *Clin Radiol* **66**, 366–382 (2011).
57 Rosenbaum SJ, Lind T, Antoch G, Bockisch A. False-positive FDG PET uptake--the role of PET/CT. *Eur Radiol* **16**, 1054–1065 (2006).
58 Massicano AVF, Marquez-Nostra BV, Lapi SE. Targeting HER2 in Nuclear Medicine for Imaging and Therapy. *Mol Imaging* **17** (2018) doi:10.1177/1536012117745386.
59 Tolmachev V, Orlova A. Affibody molecules as targeting vectors for PET imaging. *Cancers (Basel)* **12**, E651 (2020).

60 Sörensen J, Sandberg D, Sandström M, Wennborg A, Feldwisch J, Tolmachev V *et al.* First-in-human molecular imaging of HER2 expression in breast cancer metastases using the 111In-ABY-025 affibody molecule. *J Nucl Med* **55**, 730–735 (2014).
61 Sörensen J, Velikyan I, Sandberg D, Wennborg A, Feldwisch J, Tolmachev V *et al.* Measuring HER2-Receptor Expression In Metastatic Breast Cancer Using [68Ga]ABY-025 Affibody PET/CT. *Theranostics* **6**, 262–271 (2016).
62 Mortimer JE, Bading JR, Park JM, Frankel PH, Carroll MI, Tran TT *et al.* Tumor Uptake of 64Cu-DOTA-Trastuzumab in Patients with Metastatic Breast Cancer. *J Nucl Med* **59**, 38–43 (2018).
63 Gebhart G, Lamberts LE, Wimana Z, Garcia C, Emonts P, Ameye L *et al.* Molecular imaging as a tool to investigate heterogeneity of advanced HER2-positive breast cancer and to predict patient outcome under trastuzumab emtansine (T-DM1): the ZEPHIR trial. *Annals of Oncology* **27**, 619–624 (2016).
64 Ahlgren S, Orlova A, Wållberg H, Hansson M, Sandström M, Lewsley R *et al.* Targeting of HER2-Expressing Tumors Using 111In-ABY-025, a Second-Generation Affibody Molecule with a Fundamentally Reengineered Scaffold. *J Nucl Med* **51**, 1131–1138 (2010).
65 Cherry SR. Fundamentals of positron emission tomography and applications in preclinical drug development. *J Clin Pharmacol* **41**, 482–491 (2001).
66 Cherry SR, Sorenson JA, Phelps ME. Positron Emission Tomography. In: *Physics in Nuclear Medicine*. W.B. Saunders: Philadelphia, 2012, pp 307–344.
67 Strauss LG, Conti PS. The Applications of PET in Clinical Oncology. *J Nucl Med* **32**, 623–648 (1991).
68 Schomburg A, Bender H, Reichel C, Sommer T, Ruhlmann J, Kozak B *et al.* Standardized uptake values of fluorine-18 fluorodeoxyglucose: the value of different normalization orocedures. *Eur J Nucl Med* **23**, 571–574 (1996).
69 Watabe H, Ikoma Y, Kimura Y, Naganawa M, Shidahara M. PET kinetic analysis--compartmental model. *Ann Nucl Med* **20**, 583–588 (2006).
70 Hong YT, Fryer TD. Kinetic modelling using basis functions derived from two-tissue compartmental models with a plasma input function: General principle and application to [18F]fluorodeoxyglucose positron emission tomography. *NeuroImage* **51**, 164–172 (2010).
71 Wang G, Qi J. Direct Estimation of Kinetic Parametric Images for Dynamic PET. *Theranostics* **3**, 802–815 (2013).
72 Tolmachev V, Wållberg H, Sandström M, Hansson M, Wennborg A, Orlova A. Optimal specific radioactivity of anti-HER2 Affibody molecules enables discrimination between xenografts with high and low HER2 expression levels. *Eur J Nucl Med Mol Imaging* **38**, 531–539 (2011).
73 Trousil S, Hoppmann S, Nguyen Q-D, Kaliszczak M, Tomasi G, Iveson P *et al.* Positron Emission Tomography Imaging with 18F-Labeled ZHER2:2891 Affibody for Detection of HER2 Expression and Pharmacodynamic Response to HER2-Modulating Therapies. *Clin Cancer Res* **20**, 1632–1643 (2014).
74 Pai-Scherf LH, Villa J, Pearson D, Watson T, Liu E, Willingham MC *et al.* Hepatotoxicity in Cancer Patients Receiving erb-38, a Recombinant Immunotoxin That Targets the erbB2 Receptor. *Clin Cancer Res* **5**, 2311–2315 (1999).
75 Uhlén M, Fagerberg L, Hallström BM, Lindskog C, Oksvold P, Mardinoglu A *et al.* Tissue-based map of the human proteome. *Science* **347**, 1260419 (2015).
76 Houssami N, Macaskill P, Balleine RL, Bilous M, Pegram MD. HER2 discordance between primary breast cancer and its paired metastasis: tumor biology or test artefact? Insights through meta-analysis. *Breast Cancer Res Treat* **129**, 659–674 (2011).

77 Lecouvet FE, Talbot JN, Messiou C, Bourguet P, Liu Y, de Souza NM *et al.* Monitoring the response of bone metastases to treatment with magnetic resonance imaging and nuclear medicine techniques: a review and position statement by the European Organisation for Research and Treatment of Cancer imaging group. *Eur J Cancer* **50**, 2519–2531 (2014).
78 Modi S, Jacot W, Yamashita T, Sohn J, Vidal M, Tokunaga E *et al.* Trastuzumab deruxtecan in previously treated HER2-low advanced breast cancer. *N Engl J Med* **387**, 9–20 (2022).
79 Cortés J, Kim S-B, Chung W-P, Im S-A, Park YH, Hegg R *et al.* Trastuzumab deruxtecan versus trastuzumab emtansine for breast cancer. *N Engl J Med* **386**, 1143–1154 (2022).
80 Ulaner GA, Jhaveri K, Chandarlapaty S, Hatzoglou V, Riedl CC, Lewis JS *et al.* Head-to-Head Evaluation of 18F-FES and 18F-FDG PET/CT in Metastatic Invasive Lobular Breast Cancer. *J Nucl Med* **62**, 326–331 (2021).
81 McCart Reed AE, Kalinowski L, Simpson PT, Lakhani SR. Invasive lobular carcinoma of the breast: the increasing importance of this special subtype. *Breast Cancer Res* **23**, 6 (2021).
82 Lane DL, Neelapu SS, Xu G, Weaver O. COVID-19 Vaccine-Related Axillary and Cervical Lymphadenopathy in Patients with Current or Prior Breast Cancer and Other Malignancies: Cross-Sectional Imaging Findings on MRI, CT, and PET-CT. *Korean J Radiol* **22**, 1938–1945 (2021).
83 van Nijnatten TJA, Jochelson MS, Lobbes MBI. Axillary lymph node characteristics in breast cancer patients versus post-COVID-19 vaccination: Overview of current evidence per imaging modality. *Eur J Radiol* **152**, 110334 (2022).
84 McIntosh LJ, Bankier AA, Vijayaraghavan GR, Licho R, Rosen MP. COVID-19 Vaccination-Related Uptake on FDG PET/CT: An Emerging Dilemma and Suggestions for Management. *AJR Am J Roentgenol* **217**, 975–983 (2021).
85 Kubota K, Saginoya T, Ishiwata K, Nakasato T, Munechika H. [18F]FDG uptake in axillary lymph nodes and deltoid muscle after COVID-19 mRNA vaccination: a cohort study to determine incidence and contributing factors using a multivariate analysis. *Ann Nucl Med* **36**, 340–350 (2022).
86 Skawran S, Gennari AG, Dittli M, Treyer V, Muehlematter UJ, Maurer A *et al.* [18F]FDG uptake of axillary lymph nodes after COVID-19 vaccination in oncological PET/CT: frequency, intensity, and potential clinical impact. *Eur Radiol* **32**, 508–516 (2022).
87 Demmert TT, Maric I, Pomykala KL, Lueckerath K, Siveke J, Schaarschmidt BM *et al.* Novel 68Ga-FAPI PET/CT Offers Oncologic Staging Without COVID-19 Vaccine-Related Pitfalls. *J Nucl Med* **64**, 368–371 (2023).
88 Chen R, Wang Y, Zhu Y, Shi Y, Xu L, Huang G *et al.* The Added Value of 18F-FDG PET/CT Compared with 68Ga-PSMA PET/CT in Patients with Castration-Resistant Prostate Cancer. *J Nucl Med* **63**, 69–75 (2022).
89 Szigeti F, Schweighofer-Zwink G, Meissnitzer M, Hauser-Kronberger C, Hitzl W, Kunit T *et al.* Incremental Impact of [68 Ga]Ga-PSMA-11 PET/CT in Primary N and M Staging of Prostate Cancer Prior to Curative-Intent Surgery: a Prospective Clinical Trial in Comparison with mpMRI. *Mol Imaging Biol* **24**, 50–59 (2022).
90 Haug AR, Cindea-Drimus R, Auernhammer CJ, Reincke M, Wängler B, Uebleis C *et al.* The role of 68Ga-DOTATATE PET/CT in suspected neuroendocrine tumors. *J Nucl Med* **53**, 1686–1692 (2012).
91 Albanus DR, Apitzsch J, Erdem Z, Erdem O, Verburg FA, Behrendt FF *et al.* Clinical value of ^{68}Ga-DOTATATE-PET/CT compared to stand-alone contrast

enhanced CT for the detection of extra-hepatic metastases in patients with neuroendocrine tumours (NET). *Eur J Radiol* **84**, 1866–1872 (2015).
92 Kumar N, Allen KA, Riccardi D, Bercu BB, Cantor A, Minton S *et al*. Fatigue, Weight Gain, Lethargy and Amenorrhea in Breast Cancer Patients on Chemotherapy: Is Subclinical Hypothyroidism the Culprit? *Breast Cancer Res Treat* **83**, 149–159 (2004).
93 Huang J, Jin L, Ji G, Xing L, Xu C, Xiong X *et al.* Implication from thyroid function decreasing during chemotherapy in breast cancer patients: chemosensitization role of triiodothyronine. *BMC Cancer* **13**, 334 (2013).
94 Reinertsen KV, Cvancarova M, Wist E, Bjøro T, Dahl AA, Danielsen T *et al.* Thyroid function in women after multimodal treatment for breast cancer stage II/III: comparison with controls from a population sample. *Int J Radiat Oncol Biol Phys* **75**, 764–770 (2009).
95 Saga K, Jimbow K. Immunohistochemical localization of activated EGF receptor in human eccrine and apocrine sweat glands. *J Histochem Cytochem* **49**, 597–602 (2001).
96 Su Y, Zheng Y, Zheng W, Gu K, Chen Z, Li G *et al.* Distinct distribution and prognostic significance of molecular subtypes of breast cancer in Chinese women: a population-based cohort study. *BMC Cancer* **11**, 292 (2011).
97 Acheampong T, Kehm RD, Terry MB, Argov EL, Tehranifar P. Incidence Trends of Breast Cancer Molecular Subtypes by Age and Race/Ethnicity in the US From 2010 to 2016. *JAMA Netw Open* **3**, e2013226 (2020).
98 Eichler AF, Kuter I, Ryan P, Schapira L, Younger J, Henson JW. Survival in patients with brain metastases from breast cancer: the importance of HER-2 status. *Cancer* **112**, 2359–2367 (2008).
99 Boogerd W, Vos VW, Hart AA, Baris G. Brain metastases in breast cancer; natural history, prognostic factors and outcome. *J Neurooncol* **15**, 165–174 (1993).
100 Kamby C, Soerensen PS. Characteristics of patients with short and long survivals after detection of intracranial metastases from breast cancer. *J Neurooncol* **6**, 37–45 (1988).
101 Lentzsch S, Reichardt P, Weber F, Budach V, Dörken B. Brain metastases in breast cancer: prognostic factors and management. *Eur J Cancer* **35**, 580–585 (1999).
102 Kuksis M, Gao Y, Tran W, Hoey C, Kiss A, Komorowski AS *et al.* The incidence of brain metastases among patients with metastatic breast cancer: a systematic review and meta-analysis. *Neuro Oncol* **23**, 894–904 (2020).
103 Wang XY, Rosen MN, Chehade R, Sahgal A, Das S, Warner E *et al.* Analysis of Rates of Brain Metastases and Association With Breast Cancer Subtypes in Ontario, Canada. *JAMA Netw Open* **5**, e2225424 (2022).
104 Zimmer AS, Van Swearingen AED, Anders CK. HER2-positive breast cancer brain metastasis: A new and exciting landscape. *Cancer Rep (Hoboken)* **5**, e1274 (2022).
105 Stamatovic SM, Johnson AM, Keep RF, Andjelkovic AV. Junctional proteins of the blood-brain barrier: New insights into function and dysfunction. *Tissue Barriers* **4**, e1154641 (2016).
106 Bailleux C, Eberst L, Bachelot T. Treatment strategies for breast cancer brain metastases. *Br J Cancer* **124**, 142–155 (2021).
107 Solar P, Hendrych M, Barak M, Valekova H, Hermanova M, Jancalek R. Blood-Brain Barrier Alterations and Edema Formation in Different Brain Mass Lesions. *Front Cell Neurosci* **16**, 922181 (2022).

108 Park YH, Park MJ, Ji SH, Yi SY, Lim DH, Nam DH *et al.* Trastuzumab treatment improves brain metastasis outcomes through control and durable prolongation of systemic extracranial disease in HER2-overexpressing breast cancer patients. *Br J Cancer* **100**, 894–900 (2009).
109 Weigelt B, Geyer FC, Reis-Filho JS. Histological types of breast cancer: how special are they? *Mol Oncol* **4**, 192–208 (2010).
110 Franzoi MA, Hortobagyi GN. Leptomeningeal carcinomatosis in patients with breast cancer. *Crit Rev Oncol Hematol* **135**, 85–94 (2019).
111 Liu Y, Xu T, Vorobyeva A, Loftenius A, Bodenko V, Orlova A *et al.* Radionuclide Therapy of HER2-Expressing Xenografts Using [177Lu]Lu-ABY-027 Affibody Molecule Alone and in Combination with Trastuzumab. *Cancers (Basel)* **15**, 2409 (2023).

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