New approaches in the neoadjuvant systemic therapy of breast cancer

Ph.D. Thesis

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Szeged

2018

List of full papers that served as the basis of the Ph.D. thesis

I.   **Rusz O**, Vörös A, Varga Z, Kelemen G, Uhercsák G, Nikolényi A, Ormándi K, Simonka Z, Kahán Z

One-year neoadjuvant endocrine therapy in breast cancer

Pathology & Oncology Research 21:977-84 (2015)

IF: 1.940

II.   **Rusz O**+, Papp O+, Vízkeleti L, Molnár BÁ, Lotz G, Szundi Cs, Ács B, Bende KCS, Kahán Z, Székely T, Báthori Á, Kulka J, Szállási Z, Tőkés AM

LAPTM4B gene copy number gain is associated with inferior response to anthracycline-based chemotherapy in hormone receptor negative breast carcinomas

Cancer Chemotherapy and Pharmacology (2018 May)

IF: 2.808

III. Tőkés AM+, **Rusz O**+, Cserni G, Tóth E, Tőkés T, Vízkeleti L, Kószó R, Kahán Z, Kulka J, Szállási Z

Influence of mutagenic versus non-mutagenic pre-operative chemotherapy on the immune infiltration of breast cancer

Publication in progress

+equal contribution

Introduction

By use of neoadjuvant systemic therapy (NST), the radicality of surgery and radiotherapy may decrease and on the basis of therapy response the prognosis can be inferred. Over the clinical benefits, NST provides to investigate the therapy induced changes in tumor characteristics, as well as, possibilities to understand factors influencing the therapy response and survival.

In hormone receptor (HR)-positive cases the endocrine therapy is a suggested treatment, independently of the therapeutic setting (adjuvant, neoadjuvant or palliative) following chemotherapy or alone. As curative therapy, tamoxifen or the aromatase inhibitors (letrozole, anastrozole, exemestane) are accepted in combination with or without gonadotropin-releasing hormone (GnRH) agonist depending on the sex hormonal status.

From the first clinical use of endocrine therapies almost 10 years have passed until their neoadjuvant application. At the beginning only elderly women received neoadjuvant endocrine therapy (NET) with the aim of avoiding chemotherapy and its side-effect, which is poorly tolerated by this population. Nowadays, it is generally accepted that not all HR-positive cases need chemotherapy and the therapy decision should depend on tumor characteristics rather than on age. The NET should be offered in those cases, in which the best response is anticipated, mostly the Luminal A-like tumors: the typically low grade, strongly ER/PgR-positive, HER2-negative and have low proliferative fraction.

Unlike chemotherapy, ET is not able to enhance the apoptosis of cancer cells, instead, hinders or decelerates cell proliferation. Consequently, for maximum therapeutic effect, their prolonged administration is necessary, it means five or ten years in the adjuvant setting. Expanding clinical experiences indicate a pCR ratio of 0-17.5% in association with the length of NET. This ratio is surprisingly low as compared to the pCR rate of around 40% in HR-negative cases after neoadjuvant chemotherapy. Nonetheless, the long-term outcome is excellent in spite of residual cancer after NET in this group of patient. These experiences raise the question, whether pCR may be considered as a reliable end-point of treatment response in this therapeutic modality at all.

In HR-negative breast cancers the only available therapy is multicomponent chemotherapy (alone or in combination with anti-HER2 therapies based on the HER2 status). Currently, there is no existing reliable biomarker to guide the decision about the chemotherapy regimen. Anthracyclines (epirubicin, doxorubicin) are one of the most widely used agents in breast cancer. Several studies have reported that resistant cancer cells are able to accumulate

anthracyclines in the cytoplasmic organelles, resulting in reduced nuclear drug accumulation and decreased cytotoxicity. Nonetheless, the potential molecular regulation of the drug sequestration in acidic lysosomes was unclear for a long time.

In a recently published article, the amplification of 8q22 and the accompanying overexpression of the lysosomal-associated transmembrane protein 4b (LAPTM4B) was associated with worse prognosis and worse therapy response to anthracycline chemotherapy in patients with ER-negative breast cancer. LAPTM4B is a lysosomal membrane protein and its membrane-stabilizing properties are responsible for retaining the drug in the lysosome and decrease its nuclear translocation. Knockdown of *LAPTM4B* not completely but significantly weakened the capability of lysosomes to retain doxorubicin. Based on these results, LAPTM4B seems a potential biomarker to predict anthracycline sensitivity.

The complete disappearance of the tumor in HR-negative cases after neoadjuvant chemotherapy is an important surrogate end-point of favorable prognosis. Nevertheless, some patients with residual disease achieve long-term survival. Preclinical studies have suggested that chemotherapies may partly exert their antitumor activity by inducing immune response against tumor cells. This triggered immune response can keep prolonged control over the residual tumor cells. Each cytotoxic drug interacts with the immune system in its own complex manner. In addition to their direct immunomodulatory properties, the mutagenic effects of chemotherapies on tumor cells may also participate in antitumor immune activation. These acquired mutations are often translated into altered proteins including novel peptide sequences, which may become neo-epitopes. These neo-epitopes are believed to be particularly immunogenic because they are not encoded in the normal genome of the individual patient, thus reactive T-cells are not subjected to central tolerance. Recognition of neo-epitopes by cytotoxic T-cells may lead to immune-mediated tumor regression.

Previously experimentally classified chemotherapy agents may be considered as highly mutagenic (cisplatin), moderately mutagenic (cyclophosphamide) and marginally/non mutagenic (paclitaxel, doxorubicin and gemcitabine). We hypothesized that their different mutagenic properties may also contribute to different immunomodulatory effects of chemotherapies.

Aims

I. Performing a retrospective study to evaluate the benefit of one-year neoadjuvant endocrine therapy in HR-positive and HER2-negative breast carcinomas, as well as, to investigate the association between the characteristics of the residual tumor and disease outcome.

II. Examination of the *LAPTM4B* copy number by fluorescent in situ hybridization probe in breast carcinomas before treatment and its role in therapy response to anthracycline-based *versus* non-anthracycline-based therapy.

III. Comparison of the effects of preoperative therapy with the taxane-anthracycline combination (as low mutagenic regimen) *versus* cyclophosphamide-based chemotherapy (as moderately mutagenic regimen) *versus* taxane-platinum chemotherapy (as high mutagenic regimen) on the percentage of stromal tumor infiltrating lymphocytes.

Patients and methods

1.1 Study populations

One-year neoadjuvant endocrine therapy

Forty-two patients having received neoadjuvant endocrine therapy (NET) between 04/2005 and 01/2014 were included. Patients were eligible for NET if they had histologically confirmed ER- and PgR-positive, invasive breast cancer in stages II or III and imaging examinations ruled out distant metastases. Treatment with letrozole (n=33, postmenopausal group), or with goserelin plus letrozole (n=7) or with goserelin plus tamoxifen (n=2) (premenopausal group) was planned for 1 year. The therapeutic response was monitored by palpation every 3 months, or with imaging if necessary. In the event of progression, NET was replaced by neoadjuvant chemotherapy. After 1 year of NET, surgery was designed individually with regard to the post-therapy imaging results and the initial tumor stage.

On the basis of the volume of remaining tumor in surgical specimen, the following risk groups were constructed:

Group 1: no invasive tumor (stage 0)

Group 2: small-volume residual tumor (stages IA-IIA)

Group 3: large-volume residual tumor (stages IIB≤) + cases with clinical progression.

LAPTM4B gene copy number gain and response to anthracycline-based chemotherapy in hormone receptor negative breast carcinomas

A total of 143 HR-negative breast carcinoma cases were enrolled and were analyzed in two different cohorts.

The first cohort included 69 core biopsies of HR-negative (64 TNBC and 5 HER2-positive) primary breast carcinoma cases diagnosed between 2004 and 2016, who received at least two cycles of chemotherapy, and then underwent surgery. Patients were eligible for neoadjuvant therapy if they had histologically confirmed invasive breast cancer and imaging examinations ruled out distant metastases.

Fifty out of 69 patients (72.5%) were treated with anthracycline-based neoadjuvant chemotherapy, whereas 19 patients (27.5%) represented the control arm receiving non-anthracycline-based chemotherapy.

The second cohort included 74 samples of surgically removed HR-negative breast carcinomas (39 TNBC, 27 HER2-positive, and 8 with unknown HER2 data). Patients in this cohort were treated with chemotherapy in the adjuvant setting between 1999 and 2006. Out of these patients, 57/74 (77.0%) received anthracycline-based therapy and 13/74 (17.6%) received

non-anthracycline-based therapies (as control arm). In 4/74 (5.4%) cases, no treatment data were available.

Influence of mutagenic versus non-mutagenic pre-operative chemotherapy on the immune infiltration of breast cancer

Samples of 112 patients diagnosed with breast carcinoma and treated with pre-operative chemotherapy between 2005 and 2017 were selected and studied retrospectively. The inclusion criteria were as follows: availability of both a core biopsy and surgical tumor sample, known clinical and treatment data, at least 2 cycles of chemotherapy administered before surgery, residual tumor after pre-operative chemotherapy. All patients underwent breast surgery. Of the 112 cases, 103 received chemotherapy plus surgery with curative intent, while 9 cases had bone metastases at the beginning of pre-operative chemotherapy.

Based on the HR and HER2 statuses, cases were classified into four different subtypes. According to the type of pre-operative chemotherapy, the patients were grouped into platinum-based (n=28), cyclophosphamide-based (n=42) and anthracycline-based (n=42) groups.

1.2 Pathology

Histological and IHC data were collected from the original pathology reports. A case was considered HR-negative if the expression of ER and PgR was less than 1%.

The degree of pathological response to neoadjuvant therapies was categorized as followings. A complete pathological response (pCR) comprised either (i) no residual carcinoma in the breast and lymph nodes or (ii) no residual invasive tumor but DCIS present in the breast and absence of any residual invasive tumor in the lymph nodes. A partial response to therapy (pPR) meant either (i) minimal residual disease⁄near total effect (e.g. <10% of tumor remaining) or (ii) evidence of response to therapy but with 10-50% of tumor remaining or (iii) >50% of tumor cellularity remaining evident, when compared with the previous core biopsy sample, although some features of response to therapy being present. No evidence of response to therapy (pNR).

FISH analysis of LAPTM4B copy number

Interphase fluorescent in situ hybridization (FISH) analysis was used to evaluate the copy number status of *LAPTM4B* gene. Prehybridization steps was performed by ZytoLight® FISH-Tissue Implementation Kit (ZytoVision GmbH, Bremerhaven, Germany). Hybridization was carried out with custom-made, Texas Red/FITC dual labelled *LAPTM4B*/CEN8q FISH probes (Abnova Corp., Taoyuan City, Taiwan) in an automated hybridization chamber

(ZYTOMED Systems GmbH Berlin, Germany). Cell nuclei were counterstained with DAPI in antifade solution (Vector Laboratories, Inc. Burlingame, CA, 94010, USA).

Minimum two FISH images per case were digitally captured at 63x magnification by Leica DM RXA fluorescent microscope equipped with Leica DFC 365FX high performance CCD camera (Leica Microsystems GmbH, Wetzlar, Germany).

For each case, red (*LAPTM4B*) and green (CEN8 centromeric region) fluorescent signals were counted separately in at least 50 non-overlapping interphase nuclei. Based on these data the following parameters were calculated: average *LAPTM4B* copy number/cell, average CEN8q copy number/cell, *LAPTM4B*/CEN8q ratio, average *LAPTM4B* copy number/cell in amplified cell population and percentage of polysomic or amplified cells.

TIL analysis

Formalin fixed paraffin embedded blocks of core biopsies and surgical specimens were retrieved from four pathology departments (Surgical and Molecular Tumor Pathology Center, National Institute of Oncology and the Departments of Pathology, Bács-Kiskun County Teaching Hospital, Semmelweis University and University of Szeged).

4μm sections of representative tumor blocks were stained with hematoxylin and eosin. The percentage of StrTIL was evaluated according to the recommendation of International TILs Working Group 2014. Histo-pathologic evaluation of StrTILs was performed by GCs, AMT, AV, ET and JK. Controversial cases were reevaluated and discussed.

1.3 Statistical analyses

SPSS 20.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. The normality of the data was controlled by the Shapiro-Wilk test. When the data showed not normal distributions, we used non-parametric tests. For survival analyses in each study, the Kaplan-Meier method (the log-rank test) was used, whereas the hazard ratios and 95% confidence intervals (95% CI) were calculated with the Cox proportional hazard regression model.

Results

2.1 One-year neoadjuvant endocrine therapy in breast cancer

Therapeutic effects

Data of 42 patients were analyzed in the present analysis, 4 of them had bilateral breast cancer (n=46 tumors). All postmenopausal patients were treated with letrozole, while among the premenopausal patients, 7 and 2 patients received letrozole and tamoxifen in combination with goserelin, respectively. In three patients (n=4 tumors, 8.7%), the hormone therapy was changed to chemotherapy because of local progression. These patients were included in the survival analyses, but the pathological results on their surgical specimens were not used. According to the histopathological examination, all the 42 assessable tumors showed pathological response to NET. Best response, pCR was observed in 14.3% (6/42) of the tumors (6/46, 13.0 % of all tumors). In four cases, there was residual cancer neither in the breast nor in the lymph nodes while in two cases, only DCIS remained (risk group 1). Most cases (25/46, 54.3%) belonged to risk group 2, in two patients invasive residual carcinoma was detected only in the lymph nodes, and in one node-negative patient, only isolated tumor cells were found in the breast. Finally, risk group 3 comprised 11/46 (24.0%) stage IIB≤ residual tumor and 4/46 (8.7%) cases with clinical progression.

Association between tumor response to NET and tumor characteristics

A higher initial ER expression was related to a better response to NET. The likeliness of a good response to NET was increased by 7% for every 1% increase of the expression of ER (odds ratio: 1.070; 95% CI: 1.007–1.138, p=0.029). No significant associations were detected between the initial tumor grade or the expression of PgR, Ki67 or TOP2A and the therapeutic response.

The changes in ER, PgR or HER2 expression after NET were analyzed in 32 tumors since the cases that progressed (n=4) or in which there was no remaining invasive tumor in the breast (n=8) could not be included. The average expression (±SD) of ER (85.2±15.1% *vs.* 65.4±32.9%; p=0.002), PgR (66.1±32.3% *vs.* 7.7±17.7%; p=0.001), Ki67 (17.9±12.2% *vs.* 10.1±13.0%; p=0.012) and TOP2A (16.8±17.8 *vs.* 7.4±12.8; p=0.029) decreased significantly in the surgical specimens as compared with the core biopsies taken before the treatment. In one patient, the tumor completely lost both ER and PgR expression after NET. The HER2 status did not display significant changes, however in a single case, although the core biopsy indicated HER2 negativity, in the surgical specimen, IHC showed HER2 positivity, and FISH revealed the gene amplification.

Survival after NET

After a median follow-up time of 45.2 (range: 17.2-111.6) months, six patients developed distant metastases, and one patient had a second metachronous cancer in the opposite breast. Three patients died, two because of metastatic breast cancer, and one for a reason other than breast cancer. The estimated mean progression free survival (PFS) time was 74.2 (95% CI: 60.4-88.0) months and the estimated mean OS time was 92.8 (95% CI: 80.0–105.7) months. The tumor volume remaining after NET predicted PFS levels of 85.3, 70.6 and 41.4 months in risk groups 1, 2 and 3, respectively (p=0.001). The hazard ratio for PFS in groups 1 and 2 was 0.131 (95% CI: 0.016-1.056, p=0.056) and 0.101 (95% CI: 0.022-0.468, p=0.003), respectively, as compared with group 3. No significant associations were detected between the pre-treatment or post-treatment ER, PgR and TOP2A statuses or their changes and the PFS. High expression (>15%) of Ki67 in the surgical specimen predicted a risk of progression of (hazard ratio: 5.432, 95% CI: 1.202-24.553, p=0.028).

As a result of extended follow-up time (median 61.0 months, range: 17.8-136.4 months) of all patients, the estimated mean PFS was 93.0 months (95% CI: 77.0-108.9). During this period, metastases developed in 5 additional cases, hence overall 11/42 patients had distant metastases (1/5 in risk group 1; 4/23 in risk group 2; 6/14 in risk group 3); one patient had a contralateral breast cancer. Overall 13 patients died, 8 of them due to breast carcinoma, whereas 5 patients deceased of another cause.

Among those 34 patients who had been treated with sole endocrine therapy, 26 remained breast cancer-free.

Among the patients who received additional neoadjuvant (n=3) or adjuvant (n=5) chemotherapy, three cases had distant metastases and in one patient developed second breast cancer.

Updated survival analysis showed similar outcome in the 3 risk groups as earlier. There was no difference in PFS between risk group 1 and 2 (p=0.618), while the risk group 3 showed the worst outcome (estimated mean PFS, 95% CI: 113.9 months, 92.2-135.7 (risk group 1); 97.5 months, 83.2-111.8 (risk group 2); 50.9 months, 24.7-77.11 (risk group 3); p<0.001).

2.2 LAPTM4B gene copy number gain and response to anthracycline-based chemotherapy in hormone receptor negative breast carcinomas

A total of 143 cases with HR-negative breast cancer were enrolled into two cohorts.

The first cohort included 69 core biopsies of 64/69 TNBC and 5/69 HER2-positive primary breast carcinoma cases. After neoadjuvant therapy, pCR was achieved in 26 cases (37.7%), pPR in 38 cases (55.1%), and pNR in 5 cases (7.2%). The average *LAPTM4B*/CEN8q ratio was ≥2.0 in only 6/69 (8.6%) cases with the highest ratio being 3.71.

Considering the average *LAPTM4B* copy number/cell in the group of patients receiving anthracycline-based neoadjuvant therapy, higher average *LAPTM4B* gene copy number was observed in the pNR group compared to pCR group (4.1 ± 1.1 *vs.* 2.6 ± 0.1, p=0.029). We also compared average *LAPTM4B* gene copy numbers between patients who had no regression or who presented minimal response to anthracycline-based neoadjuvant therapy (>50% residual tumor remaining, pNR+pPRiii) versus cases with pCR. Again, significantly higher average gene copy number was found in the group of patients with inferior response to anthracycline-based neoadjuvant therapy (3.3 ± 0.3 *vs.* 2.6 ± 0.1, p=0.035).

The same is true for average CEN8q being significantly higher in the pNR and pNR+pPRiii groups compared to pCR group (3.7 ± 0.9 *vs.* 2.2 ± 0.1, p=0.048 and 2.9 ± 0.3 *vs.* 2.2 ± 0.1, p=0.040 respectively).

In the non-anthracycline-treated group of patients, we observed pNR in a single case. Therefore, we compared *LAPTM4B* gene copy number between pNR+pPRiii and pCR groups, resulting no significant difference (p=0.360).

Regarding average CEN8q copies, in the non-anthracycline-treated group of patients, no significant di‬were observed between pNR+pPRiii and pCR groups (p=0.879).

In the adjuvant treated cohort, 74 samples of surgically removed HR-negative breast carcinomas (39/74 TNBC, 27/74 HER2-positive and 8/74 with unknown HER2 data) were collected. During the follow-up period, distant metastases occurred in 30 (40.5%) cases. *LAPTM4B*/CEN8q ratio ≥2.0 was observed in only 4/74 (5.4%) cases. Again considering the average *LAPTM4B* gene copy number in the adjuvant anthracycline-treated patient cohort, the average *LAPTM4B* gene copy number was higher in metastatic cases, compared to the non-metastatic ones (2.2 ± 0.2 *vs.* 1.9 ± 0.1, p=0.046). In patients treated with other than anthracycline chemotherapy, no significant differences were detected between metastatic *vs.* non-metastatic groups.

Regarding average CEN8q copies, no significant differences were observed between metastatic *vs.* non-metastatic groups neither in anthracycline-treated nor in non-anthracycline-treated patients.

Comparison of the two HR-negative subtypes (HER2-positive and TNBC cases) showed no significant differences in the average *LAPTM4B* gene copy number/cell (p=0.328).

Kaplan-Meier curve estimation based on distant metastasis free survival (DMFS) revealed that higher *LAPTM4B* copy number was predictor for DMFS in the anthracycline-treated adjuvant cohort (log-rank test, p=0.037). Cut-off value for poor prognosis was defined as follows: the ratio of amplified cell population (*LAPTM4B*/CEN8q ≥2.0) is more than 15% and the average gene copy number is more than 2.5 per sample. Based on these criteria, of the 22/57 patients treated with anthracycline-based adjuvant chemotherapy and diagnosed with distant metastases, 6/22 cases presented higher *LAPTM4B* gene copy number, whereas, in 16/22 cases, lower *LAPTM4B* gene copy number was detected. Cox regression analysis was also performed, revealing association between increased *LAPTM4B* gene copy number and worse DMFS (p=0.044).

2.3 *Influence of mutagenic versus non-mutagenic pre-operative chemotherapy on the immune infiltration of breast cancer*

Samples from 112 individuals were available for analysis. The majority of patients (n=103) received neoadjuvant chemotherapy plus surgery with curative intent, while 9 women had already bone metastases at the beginning of the pre-operative chemotherapy.

Of the 28 patients undergoing platinum-based therapy, 64.3% (n=18) were HR-negative (mostly triple-negative, 46.4%, n=13), while 35.7% (n=10) were HR-positive.

Out of the 42 patients undergoing cyclophosphamide-based therapy, 23.8% (n=10) were HR-negative and 76.2% (n=32) were HR-positive.

Of the 42 patients undergoing anthracycline-based therapy, 45.2% (n=19) had HR-negative and 54.8% (n=23) had HR-positive carcinomas.

The majority of patients received more than four cycles of chemotherapy and the average cycle number was similar among the groups.

Out of the 22 HER2-positive cases, 68.2% (n=15) received pre-operative trastuzumab therapy. Trastuzumab was administered in combination with platinum-based (n=8), cyclophosphamide-based (n=5) or anthracycline-based (n=2) therapy.

StrTIL before and after chemotherapy

In the pre-treatment core biopsy samples, the median pre-StrTIL was 3.00% and more than 50% StrTIL (lymphocyte predominant) was detected in only one case. The post-StrTIL reached 50% or above in 10 cases (the pre-operative therapy was platinum-based (n=4), FEC (n=1) or docetaxel plus epirubicin (n=5).

The median post-StrTIL rose significantly to 6.25% (p<0.001) after treatment. Pre-StrTIL less than 1% was observed in 14 cases, while StrTIL less than 1% in the residual tumor occurred in only two cases.

The increase in post-StrTIL was significant both in HR-positive and HR-negative, however, in the subgroup of HR-positive/HER2-negative cases, the changes in StrTIL was significant in grade 3 cases (ΔStrTIL positive: n=14 (66.7%); zero: n=3 (14.3%); negative: n=4 (19.0%); p=0.007) but not in grade 1-2 cases (ΔStrTIL positive: n=11 (36.6%); zero: n=14 (46.7%); negative: n=5 (16.7%); p=0.075).

When analyzing the pre-StrTIL and post-StrTIL among the three treatment groups, we experienced significant StrTIL increase independently from the treatment applied.

Survival analysis according to StrTIL status

Data on DMFS was available in 103 cases. The median DMFS was 28.2 months (range: 2.6-118.3 months). Distant metastases occurred in 31/103 (30.1%) cases. In 21/31 (67.7%) cases, the primary breast carcinoma was HR-negative, and in 19/31 (61.3%) cases the post-StrTIL was lower than 10.0% or showed a decrease in comparison with the pre-StrTIL value. In univariate analyses, the HR status and the post-treatment pathological lymph node status were the only significant factors influencing DMFS. In the multivariate model changes of StrTIL showed a strong prognostic value. The Cox analysis in HR-negative cases confirmed both post-StrTIL and ΔStrTIL as playing independent prognostic role in DMFS. Each 1% increase in post-StrTIL reduced the hazard of distant metastasis development by 2.6% (hazard ratio: 0.974; 95% CI: 0.948-1.000; p=0.05) and for each 1% ΔStrTIL increment, the risk of distant metastasis was reduced by 4.3% (hazard ratio: 0.957; 95% CI: 0932-0.983; p=0.001), but according to our results, the pre-StrTIL did not influence the DMFS. The prognostic role of StrTIL in HR-positive cases could not be proven. The Kaplan-Meier analysis was carried out in HR-negative and HR-positive cases separately. Among HR-negative cases, increased or unchanged post-StrTIL was associated with better survival.

Conclusions

3.1   One-year NET results in a pCR rate of about 13% among HR-positive breast cancers. The response to NET is related to the expression of ER in the pre-therapy specimen while outcome after NET is related to the post-therapy tumor stage and Ki67 expression. The cases with stage IIB≤ residual tumor or Ki67 >15% have the worst PFS.

Long duration NET is effective and safe in cases of hormone sensitive breast cancer.

3.2   Analyzing *LAPTM4B* copy number may support future treatment decision, and the use of alternative treatment modalities without anthracyclines should be considered for those patients whose cancer harbors extra copies of *LAPTM4B.*

3.3   By comparing the effects of different preoperative chemotherapy regimens on the percentage of stromal tumor infiltrating lymphocytes resulted in no significant differences. Further investigations are warranted to clarify the mutagenicity of various chemotherapy agents and their role in induction of antitumor immune response. Post-StrTIL status and the change of StrTIL after neoadjuvant chemotherapy may be used as new prognostic factors in HR-negative breast cancer.

Acknowledgements