Quantitation of Cell Loss in Breast Cancer during Neoadjuvant Treatment (NACT) Assessed by Serum Thymidine Kinase Protein Concentration (sTK1)

Tribukait, B[1]., Jagarlamudi, K[2]., Bergh, J[1]., Hatschek, T[1]. for the PROMIX Study Group. [1]Department of Oncology/Pathology, Karolinska Institute and University Hospital, Stockholm, Sweden; [2]AroCell AB, Virdings Alle 32B, SE-754 50 Uppsala, Sweden

Background

Cell loss as a main factor in tumor growth and for response on therapy is well known for decades. Notwithstanding, to identify and quantify cell loss is still an unsolved problem.

One principal method is based on the release of intracellular macromolecules from disrupted tumor cells into the blood circulation. Here, we measured the serum concentration of thymidine kinase 1 (sTK1) from breast cancer patients during neoadjuvant chemotherapy (NATC). TK1 has a key function in DNA synthesis and repair responsible for maintaining the nucleotid pool balance by salvage and recycling thymidine from extracellular sources. Normally, TK1 is synthesized during S-phase, inactivated at mitosis and not leaked in connection with cell death. In contrast, release of TK1 by necrosis or apoptosis is typical in malignancies.

Objectives and Endpoints

To evaluate if sTK1 concentration reflects tumor response in NATC treated breast cancer patients, thus able to provide predictive and prognostic information.

Endpoints of response: Pathologic objective response
Clinical/radiological response
Endpoints for prognosis: Overall survival.

Methods

Serum from 146 patients with newly detected localized breast cancer was collected 2008-2011 during a neoadjuvant phase 2 trial (PROMIX). sTK1 (ng/ml) of the frozen serum was retrospectively mesured in duplicates by an ELISA technique, developed by AroCell (TK 210 ELISA, www.arocell.com).

Patients and Tumors

Median age was 50 years (27.8-70.6); 43% had Luminal A, 39% Luminal B and 18% TNBC tumors (PAM 50). Median tumor diameter was 55 mm (20-180 mm). Axillary nodes were present in 72%, other regional nodes in 14%. Median Ki67/Mib1 LI was 30% (1-90%).

Treatment, Clinical/Radiological Evaluations, sTK1 Analyses (Fig 1).

All patients received 6 courses Epirubicin+Docetaxel every 3 weeks. Following the 1st clinical/radiological evaluation after 2 courses, Bevacizumab was added to the remaining 4 courses. Four patients with progressive disease were individually treated.

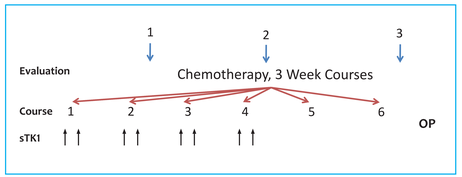

Fig 1. ***Schedule of Chemotherapy, Clinical/Radiological Evaluations and sTK1 Measurements in 146 Neoadjuvant Treated Breast Cancer Patients***

Results

sTK1 Concentration before and 48h after Treatments (Fig 2).

Mean base-line sTK1 concentration was 0.34 ng/ml (range 0.1-1.29 ng/ml).

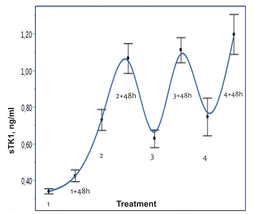

Fig 2. ***sTK1(ng/ml) before and 48h after Treatment 1, 2, 3 and 4. Mean Values ± SE***

Pathologic Objective Response and sTK1 before Treatments (Fig 3 a, b).

Pathologic findings (irrespective of node status) after 6 courses of chemotherapy were: pT0 20.6% (n=30), pT1 35.6% (n=52), pT2 21.9% (n=32), pT3 13% (n=19), pT4 0.7% (n=1), pTx 8.2% (n=12).
sTK1 was studied before treatments in responding and non-responding patients (Fig 3a), in (Fig 3b) further subdivided according to stage.

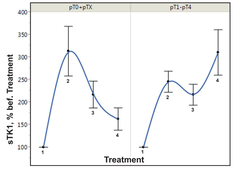

Fig 3a.
sTK1 in % (Base-line = 100%) before Treatment 2, 3 and 4 in Responding and Non-responding Patients. Mean Values ± SE

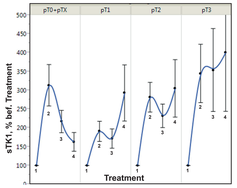

Fig 3b.
Non-responding Patients were subdivided according to Stage. A stage dependent increase in sTK1 can be observed.

Pathologic Objective Response and sTK1 *48h after* Treaments (Fig 5).

sTK1 48h after treaments 2, 3 and 4 ranged between 2 and 3.5 times base-line values, different in pT0 + pTx, pT1, pT2 and pT3 and independent on their corresponding values before treatment.

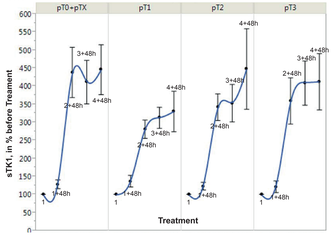

Fig 5.
sTK1 in % (Base-line=100%) 48h after Treament 2, 3 and 4 in Responding and Non-responding Patients

Suvival and sTK1 48h after Treatments (Fig 6).

After a median follow-up of 60.3 months (6.9 - 72.1) 30/126 of the patients had deceased from breast cancer; of pT1, pT2, pT3+4 and pT0+pTx in 9.6%, 15.6%, 30% and 33.3%, respectively.
A relationship was found between stage related survival and sTK1 48h plateau values after treament 2, 3 and 4.

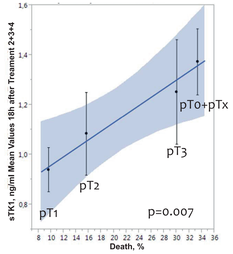

Fig 6. ***Breast Cancer Specific Mortality (%) at 60 Months Follow-up in Patients with pT1, pT2, pT3 and pT0+pTx Tumors vs. Means (± SE) of sTK1 48 h after Treatment 2, 3 and 4.***

sTK1 before Treatments in Relation to Tumor Volume (Fig 4).

Initial tumor volume and volumes at the 1st and 2nd response evaluation were calculated from the tumor diameters. Medium base-line volume was 87 cm³ (4-3050 cm³). sTK1 was related to tumor volume in responding and non-responding patients.

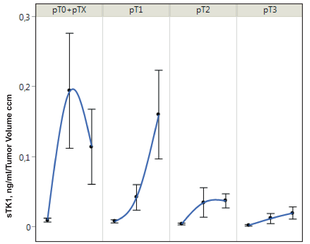

Fig 4. ***sTK1, ng/ml per Tumor Volume, cm³ in Responding, and Non-responding Patients before Treatment and at the 1st and 2nd Response Evaluation. Mean Values ± SE***

Tumor Response during Therapy (Fig 7).

Tumor response was evaluated after Treatment 2, 4 and 6 by clinical/radiological methods. After 6 treatment cycles complete response in the breast was achieved in 30%, partial response in 60% and no effect in 10% of the patients.

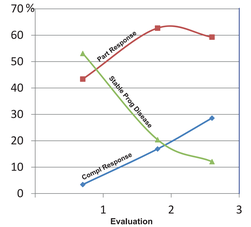

Fig 7. ***Complete Response, Partial Response and Stable/Progressive Disease in 146 Patients during Preoperative Chemotherapy***

Clinical /Radiological Response Evaluations and sTK1 before Treatments (Fig 8a, b).

At the 1st response evaluation no difference in sTK1 was found between non-responding and responding patients. At the 2nd and 3rd response evaluation, sTK1 changes measured before treaments were associated with particular tumor reactions.

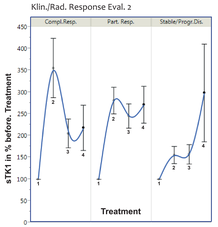

Fig 8a.
Clinical/Radiological Response Evaluation 2.

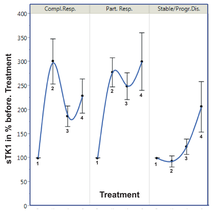

Fig 8b.
Clinical/Radiological Response Evaluation 3. sTK1 in % (Base-line = 100%) before Treatment 2, 3 and 4 in Patients with Complete Response, Partial Response and Stable/Progressive Disease.
Mean Values ± SE.

Discussion and Conclusions

Use of blood based markers is non-invasive and convenient, can easily be repeated, comprises both the primary tumor and tumor deposits, and is of comparably low cost. The acceptance of serum biomarkers for clinical applications depends on validation studies with established endpoints.

In this study we made use of NACT and the possibility to directly observe treatment response in breast cancer patients with pathologic objective response and clinical/radiological response evaluations as predictive endpoints and, due to the retrospective character of the investigation, overall survival as prognostic endpoint.

Our main conclusion is that measurement of sTK1 in blood is a valid marker for tumor cell loss during NACT.

Measurement of sTK1 concentration before the courses of treatment enable to follow the tumor reaction on therapy, supporting and enhancing the information of tumor size measurements by combined physical examinations, mammography, ultrasound and MRI. Conspicuous differences in sTK1 levels between responding and non-responding patients appear after the 2nd course of therapy.

The significance of tumor size for cell loss by chemotherapy is an important aspect in the treatment of tumors.

High sTK1 concentrations 48h after chemotherapy also in the absence of primary tumor, and their association with survival was unexpected and may indicate drug induced cell mobilization and release from occult metastatic deposits.

Monitoring cancer reaction during NACT is essential in individualized therapy and drug development; sTK1 measurements might also be useful in adjuvant settings of therapy, in malignancies not directly accessible for observation and as an adjunct to other serum markers.

bernhard.tribukait@ki.se