Tumour Response to Neoadjuvant Chemotherapy in Breast Cancer: Routine Pathologic Markers Improve the Predictive Power of a Cell-Loss Metric Based on Release of Thymidine Kinase 1 (sTK1) into Blood.

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Background

Early prediction of tumour response to therapy is essential for individualized treatment and sparing non-responders needless harm.

In neoadjuvant chemotherapy of breast cancer, a positive relationship has recently been found between an early measure of tumour cell loss and pathologic response at surgery.

The "cell-loss metric" was based on the serum concentration of thymidine kinase 1 (sTK1) – a macromolecule released when proliferating tumour cells are disrupted – and tumour volume.

Pathologic complete response (pCR) was more likely in patients with a high cell-loss metric.

Objectives and Endpoint (Fig. 1)

1. To investigate how the predictive power of the cell-loss metric can be improved by combining it with other basic tumour characteristics.
2. To optimize the time point for application of the cell-loss metric by comparing its performance prior to and 48h after the 2nd course of chemotherapy, i.e. in conjunction with a marked increase in sTK1.

Endpoint: Local tumour response at surgery after 6 courses of therapy.

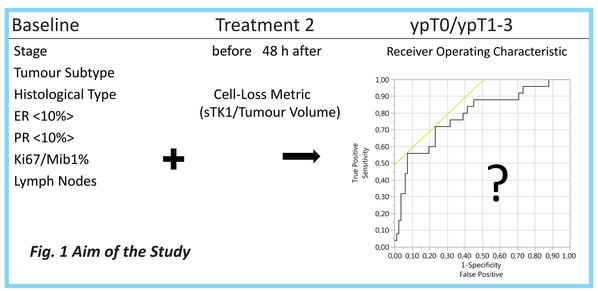

Fig. 1 Aim of the Study

Methods

Serum from 146 patients with newly detected, localized, non-metastatic BC was collected 2008-2011 during a neoadjuvant phase 2 trial (Promix, Clinical Trial gov. NET 000957125). TK1 of the frozen serum was measured retrospectively in duplicates by an ELISA technique, developed by AroCell (TK 210 ELISA, www.arocell.com).

The present analysis comprised 58 patients, from whom serum had been obtained prior to and 48h after treatment 1, 2, 3 and 4. Tumour volume was calculated from the largest diameter of the tumour, measured at baseline (BL), after treatment 2, 4 and 6 **(Fig. 2)**.

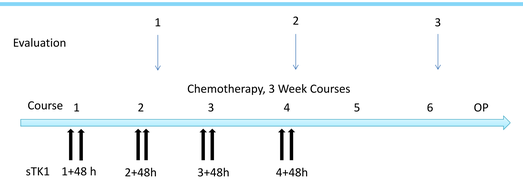

Fig. 2 Schedule for Chemotherapy, Clinical/Radiological Evaluations and sTK1 in 58 BC Patients undergoing Neoadjuvant Treatment.

Patients, Tumours and Treatment

Median age was 49 (33-66) years; 39% of the patients had Luminal A, 33% Luminal B and 28% TNBC tumours. 64% had positive axillary lymph nodes. Median BL tumour diameter was 58 (20-180) mm, median Ki67/Mib1 LI was 30 (1-90) %. The patients received neoadjuvant epirubicin/docetaxel (75 mg/m² i.v. each) in 6 cycles every 3 weeks, supplemented by bevacizumab (15mg/kg i.v.) on day 1 of cycle 3-6 **(Fig. 2)**.

Results

sTK1 median BL was 0.28 (range 0.1-0.72) ng/ml. It had increased two-fold prior to treatment 2-4 and three-fold 48h after treatments **(Fig. 3)**.

Tumour volume decreased exponentially during treatment **(Fig. 4)**.

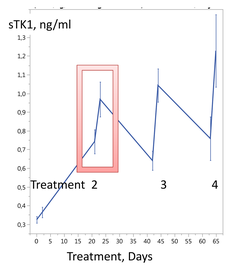

Fig. 3 sTk1 Concentration, ng/ml, during Treatment. Mean Values ± SE

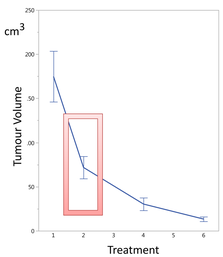

Fig. 4 Tumour volume during Treatment

Local tumor control was reached in 15/58 (26%) of patients and was related to intrinsic subtypes (p=0.03), **(Fig. 5)**.

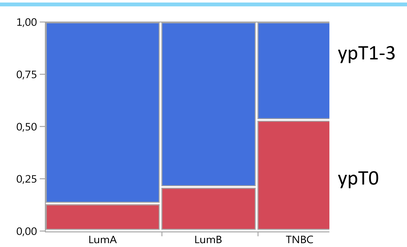

Fig. 5 Tumour Response after 6 Cycles of Therapy

Median cell-loss metric BL was 0.0032 (0.0001-0.0693) ngxml-1/cm³. Prior to and 48h after treatment 2 the level was increased by respectively 5 and 7 times. For these time points there was a significant difference between patients who reached local tumour control (ypT0) and those with remaining tumour (ypT1-3). **(Fig. 6)**.

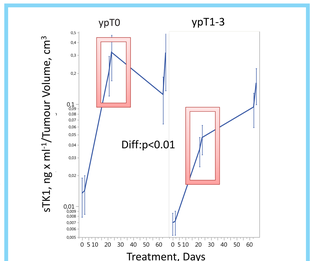

Fig. 6 ***Cell loss Metric at treatment 2 and 4 in Relation to Tumour Response. Mean values ± SE***

Independent patient and tumour characteristics at BL were: age, menopausal status, axillary lymph nodes, estrogen, progesterone and HER2 receptor status, histological type, luminal A , luminal B and TNBC subtype, Ki67/Mib1 LI, sTK1 concentration. Forward selection revealed that in addition to the cell-loss metric at T2 (sTk1 T2/ tumour volume) estrogen receptor status (ER <10> %) and histological tumour type (lobular-ductal & other) were the most significant factors. These were incorporated into a logistic regression algorithm and their contribution to tumour response analysed by ROC analysis **(Fig. 7 a-c)**. The AUC increased by addition of ER and histological type from 0.703 to 0.789 and 0.843, respectively. By adding the ratio between Ki67 and tumour volume (Ki67/tumor volume;BL) and PR (<10%>) sensitivity of 0.93 at a specificity of 0.975 (AUC 0.916) was achieved **(Fig. 7 d)**.

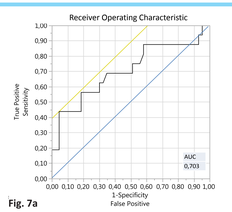

Fig. 7a

Source	Nparm	DF	L-R ChiSquare	Prob>ChiSq
sTK1 T2/Tumour Volume T2	1	1	8,84061647	0,0025*

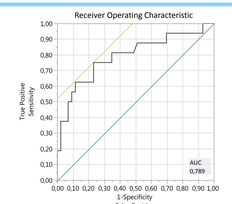

Fig. 7b

Source	LogWorth	PValue
Estrogen Receptor <10%>	1,889	0,01291
sTK1 T2/Tumour Volume T2	1,885	0,01305

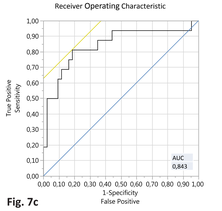

Fig. 7c

Source	LogWorth	PValue
sTK1 T2/Tumour Volume T2	2,359	0,00438
ER BL <10>	2,208	0,00619
Histological Type (Lobular-Other+Ductal)	1,533	0,02933

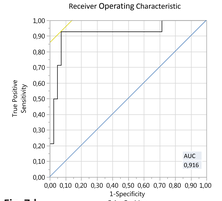

Fig. 7d

Source	LogWorth	PValue
sTK1 T2/Tumour Volume T2	4,607	0,00002
PR BL <10>	2,770	0,00170
Ki67BL/Tumour Volume BL	2,382	0,00415
Histological Type	0,516	0,30465

sTK1 concentrations before and 48h after treatment 2 were 0.75 and 0.97 ng/ml, and corresponding cell-loss metrics 0.0243 and 0.1174 units, respectively.

Generally, the predictive power of the cell-loss metric was higher when pre-treatment values were used **(Fig. 8)**.

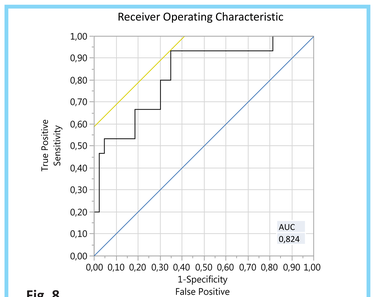

Fig. 8

Source	LogWorth	PValue
A) TK1 protein Treatment 2+48h/Tumor Vol T2	2,415	0,00385
ER BL <10>	2,198	0,00634
Histological type	0,830	0,14776

Discussion and Conclusions

The usefulness of data on macromolecules released from tumour tissue into blood circulation can be improved by combining them with other tumour properties. Thus, during neoadjuvant treatment of breast cancer, serum levels of thymidine kinase 1 was related to tumour volume. This cell-loss metric appears useful for early prediction of pathological response as established at surgery. Here we could demonstrate that the predictive power of the metric was significantly improved by adding tumour markers such as hormone receptor status and tumour origin (lobular or ductal) obtained by routine histo-pathology at diagnosis of the tumour. The time point 3 weeks after the first course of chemotherapy, i.e. prior to the 2nd course, appears to be most appropriate for analysis. Early prediction of tumour response makes the cell-loss metric potentially useful in personalized oncology and in the evaluation of new therapeutic modalities.

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