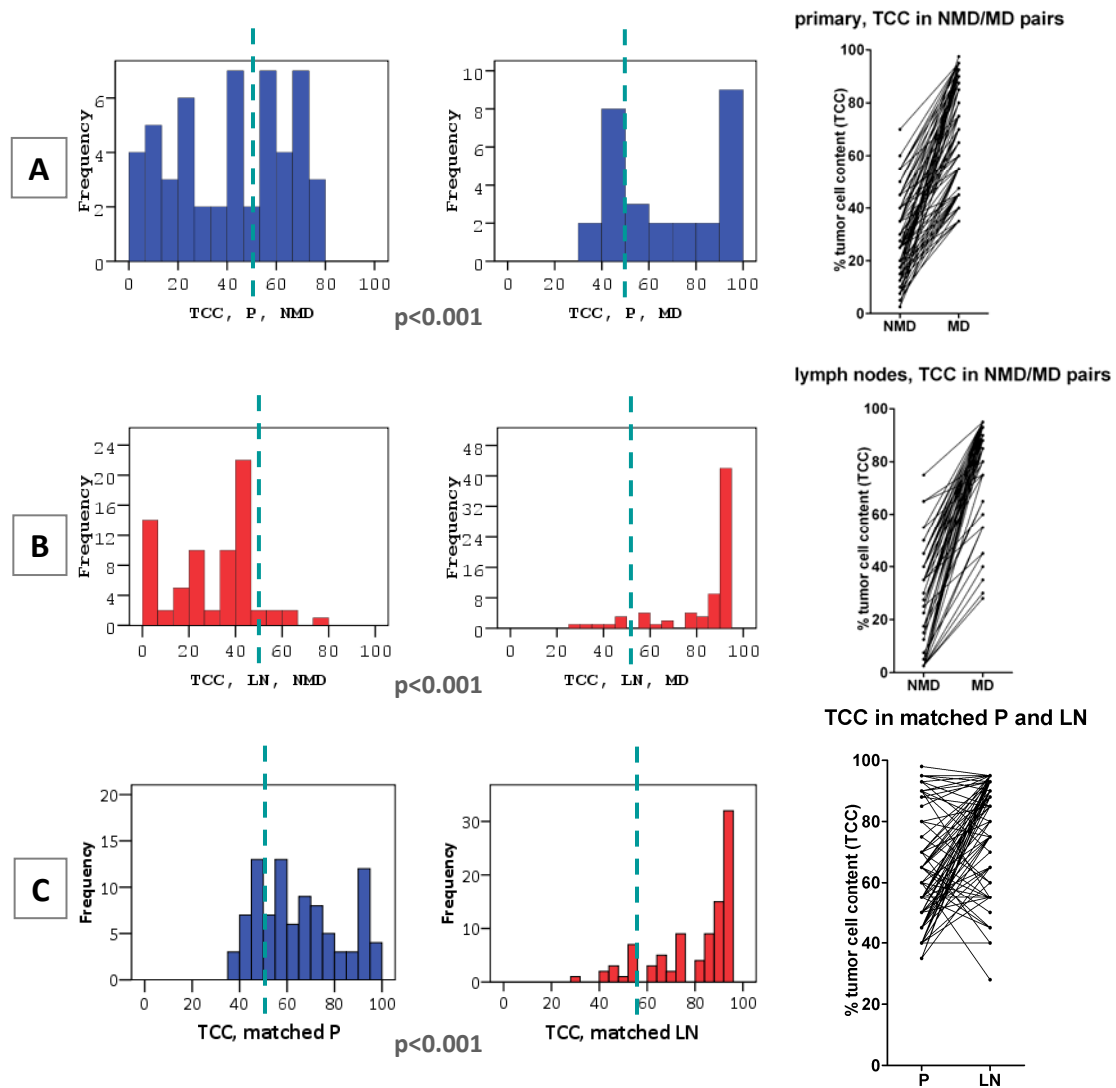


Sample parameters affecting the clinical relevance of RNA biomarkers in translational breast cancer research

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ESM_2: TUMOR CELL CONTENT IN PAIRED SAMPLE SERIES

Figure ESM_2_1



ESM_2_1: Tumour cell content (TCC%) distribution in the three matched sample series examined. A, matched non-macrodissected (NMD) and macrodissected (MD) primary tumours (P); B, matched NMD/MD metastatic lymph nodes (LN); C, matched P/LN. TCC values (x axes in A, B, C) are percentages (%). Green dashed lines were set at 50% TCC. Wilcoxon Signed Ranks Test results for paired samples are shown. TCC ratios upon MD were higher than 50% in 72 (73.5%) and higher than 75% in 44 (44.9%) out of 98 matched P samples. The same TCC ratios were achieved in 65 (90.3%) and 58 (80.6%) out of 72 matched LN samples. In

the mP / mLN series, TCC was higher than 50% in 75.3% of mP and 93.5% of mLN samples, similar to those in the corresponding P-MD and LN-MD groups (compare TCC matched P with TCC P, MD, and TCC matched LN with TCC LN, MD graphs). As expected, TCC% differed in both directions (higher and lower) between mP and mLN paired samples (C, plotted RQ values).

Figure ESM_2_2

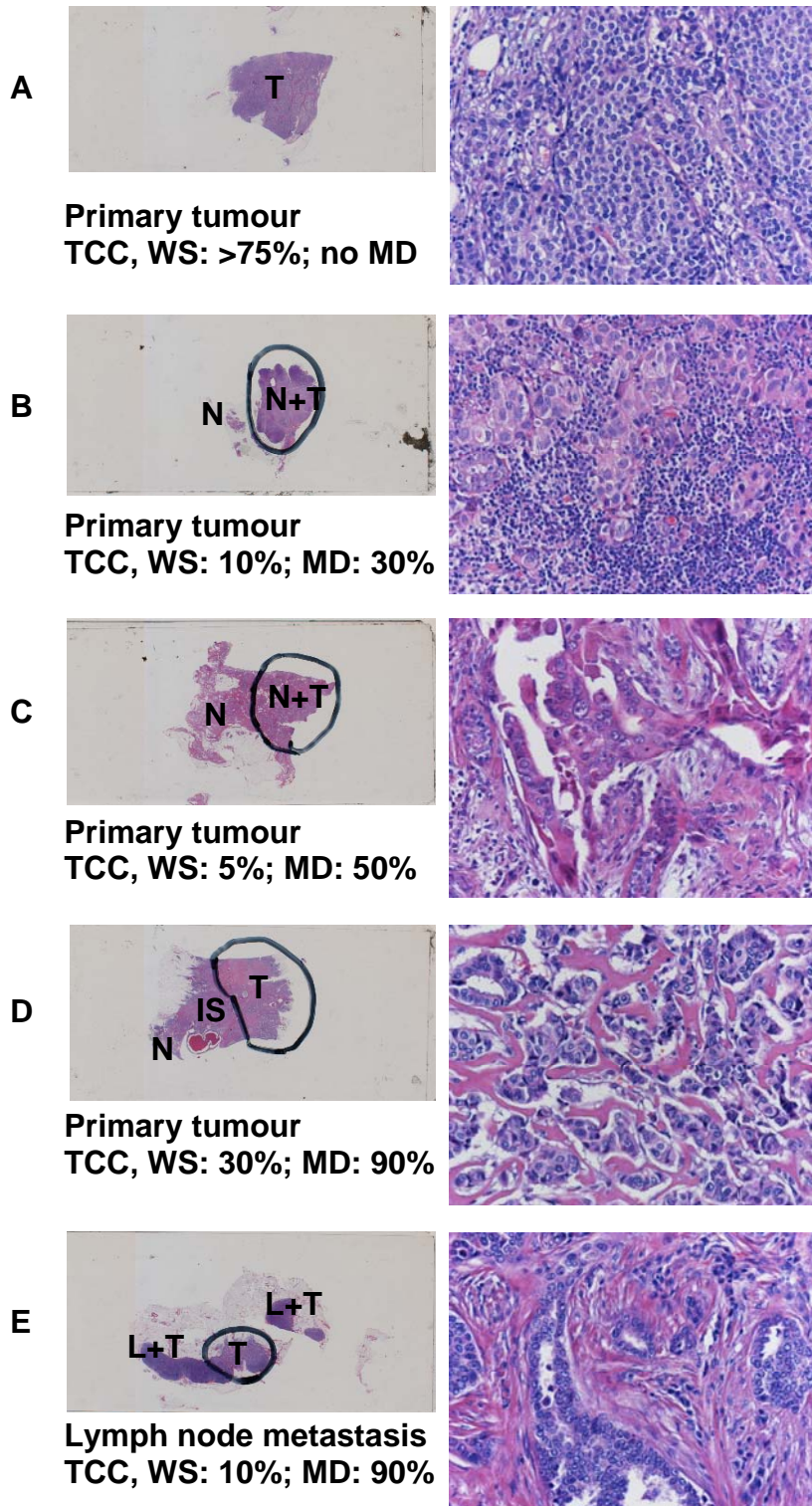


FIGURE ESM_2_2: Macrodissection of primary tumours may not yield >70% TCC in molecular samples. Case A, an almost homogeneous primary tumour in this section with >75% tumour cells did not necessitate macrodissection (MD); this case is

shown in comparison to cases B – E exhibiting low TCC in the whole section (WS). Dense lymphocytic infiltrates (in B) or mixed normal and tumour elements (in C) result in higher TCC% in MD samples than in the non-macrodissected WSs, but the achieved percent is still low. The metastatic lymph node shown is representative for the majority of tissue sections in this group. Cases D and E can be considered as efficient for macrodissection, which is the rule for lymph node metastases but not for primary tumours (compare D with B and C). N: normal breast; IS: in situ carcinoma; T: tumour; L: lymph node. Note that tumour stroma and therein hosted cells are always included in the macrodissected area and, hence, in the MD samples.