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RNA Sequencing-Based Single Sample Predictors of Molecular Subtype and Risk of Recurrence for Clinical Assessment of Early-Stage Breast Cancer.

52P

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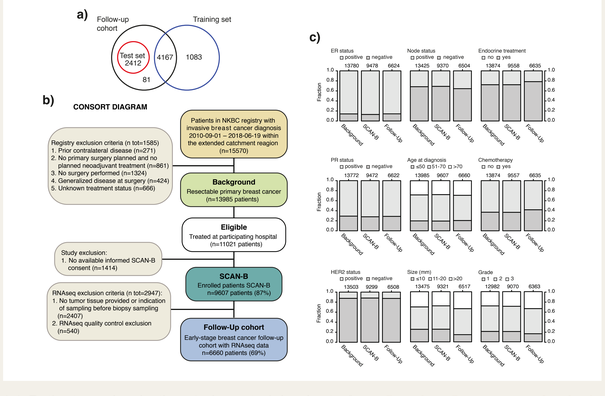
Conclusion

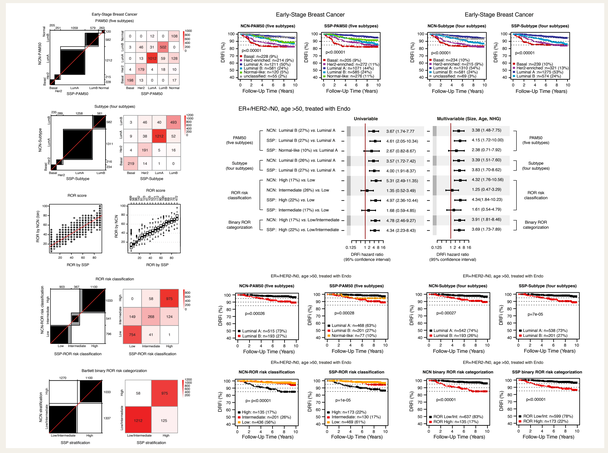
Single-sample predictor (SSP) models utilizing RNA-sequencing (RNAseq) data can be derived to closely match nearest-centroid (NC) models and clinical tests. Agreement and outcome analyses suggest that NC and SSP models are interchangeable on a group-level. Retrospective evaluation in postmenopausal ER+/HER2-/N0 breast cancer suggested that molecular testing could change therapy recommendation for up to one-fifth of patients with balanced escalation and de-escalation of chemotherapy.

Background

Multigene expression assays for molecular subtypes and biomarkers can aid clinical management of early-stage invasive breast cancer. Based on RNAseq we aimed to develop robust SSP models for conventional clinical markers as well as molecular intrinsic subtype and risk of recurrence (ROR) that provide clinically relevant prognostic stratification.



(**a**) Patient overlap in the study material cohorts from **Sweden Cancerome Analysis Network Breast (SCANB)** and **(b)** consort diagram of patient selection for the early-stage follow-up subcohort with **(c)** bar charts illustrating population-based representativeness compared to the background population. **SCANB** is an ongoing population-based observational study (ClinicalTrials.gov identifier: NCT02306096) with long-term translational goals for improved diagnostics, prognostics and treatment prediction in breast cancer. Inclusion rates are high and >85% of the eligible catchment population is enrolled. The SCANB material represents a unique population-based representation of contemporary primary breast cancer. Between 2010 and 2022 more than **18000 patients** have enrolled and over **13500 tumor samples** have been collected and processed for RNA sequencing.



Validation of SSP vs. NC classifications in the independent test set of early breast cancer. Agreement chart and confusion matrix comparing SSP (x-axis/columns) with NCN (y-axis/rows). Scatterplot of binned ROR values and boxplot of ROR values by NCN (y-axis) vs. SSP (x-axis). **Assessment of prognostic value of SSP stratification and NCN stratification.** Kaplan-Maier plots and Forest plots of Hazard ratios and 95% CI from Cox regression for SSP and NCN stratification in the independent test set.

Methods

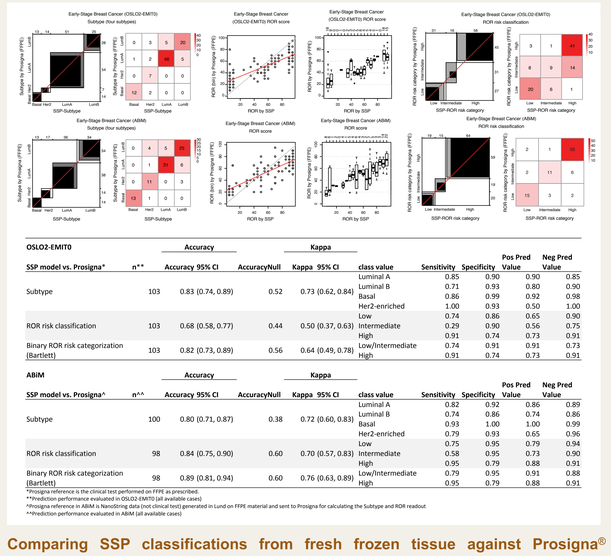
A uniformly accrued **SCANB** breast cancer cohort of 7743 patients with RNAseq data from fresh tissue was divided into training set and test set. We trained SSPs for PAM50 subtypes and ROR assigned by consensus NC models (NCN) and for conventional clinical markers from histopathology. Agreement and prognostic value was assessed in the test set. SSPs were compared with Prosigna® in two external cohorts. SSP classifications were also investigated in the entire SCANB follow-up cohort of 6660 patients with early-stage breast cancer.

		Accuracy			Kappa					
SSP model	n	Accuracy	95% CI	AccuracyNull	Kappa	95% CI	class value	Sensitivity	Specificity	Pos Pred Value	Neg Pred Value
ER	2410	0.96	(0.96, 0.97)	0.86	0.86	(0.83, 0.89)	Positive	0.97	0.96	0.99	0.82
PR	2409	0.87	(0.86, 0.89)	0.73	0.70	(0.67, 0.73)	Positive	0.88	0.87	0.95	0.72
HER2	2410	0.89	(0.87, 0.90)	0.88	0.58	(0.54, 0.62)	Positive	0.87	0.89	0.51	0.98
HER2 (SSP ER specific)	2410	0.92	(0.91, 0.93)	0.88	0.67	(0.62, 0.71)	Positive	0.86	0.93	0.60	0.98
Ki67	900	0.80	(0.77, 0.82)	0.57	0.59	(0.53, 0.64)	High	0.80	0.79	0.83	0.75
							Grade 1	0.81	0.72	0.35	0.95
NHG	2357	0.57	(0.55, 0.59)	0.49	0.38	(0.35, 0.40)	Grade 2	0.30	0.88	0.71	0.57
							Grade 3	0.84	0.79	0.69	0.90

Validation of SSP models for clinical markers in the independent test set.

Results

Agreement between SSP and NC classifications for Subtype was very high (90%, Kappa=0.84). Accuracy for ROR risk category was high (84%, Kappa=0.75, weighted Kappa=0.90). Prognostic value for SSP and NC was assessed as equivalent. Agreement for SSP and histopathology was very high or high for receptor status, while moderate and poor for Ki67 status and Nottingham histological grade. SSP concordance with Prosigna® was high for subtype and moderate and high for ROR risk category. In pooled analysis, concordance between SSP and Prosigna® for emulated treatment recommendation for chemotherapy (yes *vs.* no) was high (85%, Kappa=0.66). In postmenopausal ER+/HER2-/N0 patients SSP application suggested changed treatment recommendations for up to 17% of patients, with balanced escalation and de-escalation of chemotherapy.

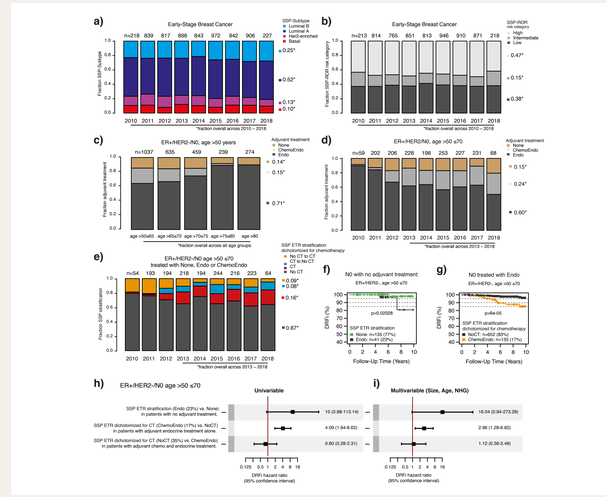


OSLO2-EMIT0		Accuracy			Kappa					
SSP model vs. Prosigna*	n**	Accuracy	95% CI	AccuracyNull	Kappa	95% CI	class value	Sensitivity	Specificity	Pos Pred Value	Neg Pred Value
							Luminal A	0.85	0.90	0.90	0.85
Subtype	103	0.83	(0.74, 0.89)	0.52	0.73	(0.62, 0.84)	Luminal B	0.71	0.93	0.80	0.90
							Basal	0.86	0.99	0.92	0.98
							Her2-enriched	1.00	0.93	0.50	1.00
							Low	0.74	0.86	0.65	0.90
ROR risk classification	103	0.68	(0.58, 0.77)	0.44	0.50	(0.37, 0.63)	Intermediate	0.29	0.90	0.56	0.75
							High	0.91	0.74	0.73	0.91
Binary ROR risk categorization (Bartlett)	103	0.82	(0.73, 0.89)	0.56	0.64	(0.49, 0.78)	Low/Intermediate	0.74	0.91	0.91	0.73
							High	0.91	0.74	0.73	0.91

ABiM		Accuracy			Kappa					
SSP model vs. Prosigna^	n^^	Accuracy	95% CI	AccuracyNull	Kappa	95% CI	class value	Sensitivity	Specificity	Pos Pred Value	Neg Pred Value
							Luminal A	0.82	0.92	0.86	0.89
Subtype	100	0.80	(0.71, 0.87)	0.38	0.72	(0.60, 0.83)	Luminal B	0.74	0.86	0.74	0.86
							Basal	0.93	1.00	1.00	0.99
							Her2-enriched	0.79	0.93	0.65	0.96
							Low	0.75	0.95	0.79	0.94
ROR risk classification	98	0.84	(0.75, 0.90)	0.60	0.70	(0.57, 0.83)	Intermediate	0.58	0.95	0.73	0.90
							High	0.95	0.79	0.88	0.91
Binary ROR risk categorization (Bartlett)	98	0.89	(0.81, 0.94)	0.60	0.76	(0.63, 0.89)	Low/Intermediate	0.79	0.95	0.91	0.88
							High	0.95	0.79	0.88	0.91

*Prosigna reference is the clinical test performed on FFPE as prescribed.
**Prediction performance evaluated in OSLO2-EMIT0 (all available cases)
^Prosigna reference in ABiM is NanoString data (root clinical test) generated in Lund on FFPE material and sent to Prosigna for calculating the Subtype and ROR readout
^^Prediction performance evaluated in ABiM (all available cases)

Comparing SSP classifications from fresh frozen tissue against Prosigna® classifications from FFPE tissue in two external cohorts. Agreement chart and confusion matrix for SSP (x-axis/columns) vs. Prosigna® (y-axis/rows). Scatterplot of binned ROR values and boxplot of ROR values by Prosigna® (y-axis) vs. SSP (x-axis). OSLO2-EMIT0 cohort (top charts and table) and ABiM cohort (bottom charts and table).



SSP classifications for Subtype and ROR risk category in the extended SCANB cohort of early-stage breast cancer and cross-comparison with administered systemic treatment. Proportions by year of diagnosis: (**a**) SSP-Subtype (**b**) SSP-ROR risk category. (**c**) Proportions for adjuvant treatment within ER+/HER2-/N0 patients age >50 years by age at diagnosis. (**d**) Proportions for adjuvant treatment within ER+/HER2-/N0 patients diagnosed at age >50 ≤70 years by year of diagnosis. (**e**) Cross-comparison of a naïve SSP emulated treatment recommendation dichotomized for chemotherapy (CT) (yes *vs.* no) with records of administered systemic treatment. SSP treatment recommendation in agreement with administered treatment are shown in black (No CT) and in red (CT). The discordant groups are shown in orange (No CT to CT) and in blue (CT to No CT). Kaplan-Maier plot for SSP stratification within (**f**) subgroup with no adjuvant treatment and (**g**) subgroup treated with adjuvant endocrine therapy only. Forest plots of Hazard ratios and 95% CI from (**h**) univariable and **(I)** multivariable Cox regression.

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