Appendix "Supplementary material"

High PDGFRb expression predicts resistance to radiotherapy in DCIS within the SweDCIS randomized trial.

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Supplementary Material and Methods

Marker selection and previous markers tested in the SweDCIS cohort

Stromal PDGFRb expression was predefined to be assessed as a single marker. The marker selection hypothesis-driven selection following earlier findings of survival-association-trends of this marker in DCIS (1) and premenopausal breast cancer patients (2), as well as its predictive capacity for tamoxifen benefit (3).

No other stroma markers were stained and assessed in the SweDCIS material by the authors, the Swedish SweDCIS trial group or others. In the five earlier, independent publications by the Swedish SweDCIS trial group no immunohistochemistry data were used (4–8). The SweDCIS material was further used in an independent validation study of the DCISionRT test/score (PreludeDx, CA, USA), which includes a combination of seven protein tumor markers (PR, Ki67, HER2, FOXA1, p16/INKA4, COX2 and SIAH2) and four clinical factors (age, size, margin and palpability). The preliminary data of this study was presented at the 2017 San Antonio Breast Cancer Symposium (abstract GS5-08, 8th Dec 2017). According to these preliminary data, although weak trends were noted for some of the individual different markers towards a predictive value for RT benefit, none of the individual different markers predicted sensitivity to RT with a statistical significance as determined by formal interaction tests between RT and the markers.

Immunohistochemistry

Immunohistochemical staining (IHC) was performed for PDGFRb on 4 um whole sections from archived formalin-fixed paraffin embedded tissue block using a Ventana autostainer (Ventana Benchmark Discovery, NexES_V10.6) and the rabbit monoclonal anti-PDGFRb

antibody (clone 28E1, #3169 CellSignaling, Danvers MA, US; 1:100 dilution) diluted in Antibody Diluent Buffer (Antibody diluent, Ventana, Tuscon, Arizona, US). The staining protocol was performed with the Discovery OmniMap anti-rabbit HRP kit (Ventana) and included an extended antigen retrieval step with pH10 Tris buffer (Sigma-Aldrich and Merck Kgaa, Darmstadt, Germany), primary antibody incubation for 1 h and secondary antibody for 32 min. Normal breast tissue was used as positive control. Potential cross-reactivity of the PDGFRb antibody with the structural related PDGFRa was excluded as described previously (1).

PDGFRb scoring

Scoring was performed by two independent raters (CS, DF), under guidance of an experienced breast pathologist (LAA) and blinded to clinical and outcome information. Each rater assigned ten high power fields (0.25mm^2) per patient section, located within the DCIS associated stroma region, and scored each high power field for average intensity of the stromal PDGFRb staining following a four-grade scale: none/negative (0+), weak (1+), moderate (2+), strong (3+) (Supplementary Figure 1A). For each rater the average PDGFRb score based on all assigned high-power fields per patient was calculated (Supplementary Figure 1B) and the degree that raters provided consistency in their scorings was assessed by the equally weighted Cohen's kappa coefficient (κ) as well as the interclass Correlation Coefficient (with model="twoway", type="agreement", unit="single") using the *irr* package for R (9) (Supplementary Figure 1B). The mean scores between the raters were used in subsequent statistical analyses (Supplementary Figure 1C).

The PDGFRb scoring data was split at the median of the distribution to obtain two patient groups as equally sized as possible, referred to as PDGFRb^{low} and PDGFRb^{high}-group (Supplementary Figure 1C).

In about 30% of cases the DCIS area was too small to assign a total of ten high power fields, in these cases the maximum possible number of high-power fields were recorded. Cases with less than three available high-power fields were excluded. In rare cases where the average scores of the raters had a difference larger than two grades were reviewed to exclude technical errors and corrected if needed.

Additional scoring approaches

Two additional scoring approaches were also used, both relying on scoring by two raters (CS, DF). One approach, referred to as hot spot scoring, assessed the average stromal PDGFRb staining intensity in the high-power field where tumor-associated stroma displayed highest PDGFRb expression. The other scoring approach is described in ((2)) and integrates the overall average stromal PDGFRb staining intensity weighted by the general stroma abundance in the whole tumor area and is referred to as integrated overall scoring approach. Both approaches used the same four-grade scale and dichotomization strategies as described in the scoring approach based on multiple vision fields.

Additional clinico-pathological characteristics

Data on clinoco-pathological factors (age, tumor size, focality, endocrine therapy, surgical margin status *etc*.) were collected from the SweDCIS trial data-base. Nuclear grade, KI67 and tumor infiltrating lymphocytes was added after external, central pathologists review.

Nuclear grade was evaluated based on a separate Hematoxylin/Eosin staining. Low nuclear grade/1 was defined as DCIS cell nuclei that were small with minimal size variation and dense nuclear chromatin. Intermediate nuclear grade/2 was defined as having moderate nuclear pleomorphism (variation is size/shape), with mild to moderately open chromatin, small inconspicuous nucleoli and rare mitotic figures. High nuclear grade/3 shows moderate to marked nuclear pleomorphism, moderate to marked open chromatin with prominent nucleoli and increased mitotic figures. KI67 index was obtained from KI67 IHC on a separate section and the upper quartile (≥ 20% KI67 positive nuclei) was defined as the patient group with high KI67 index. Tumor infiltrating lymphocytes in DCIS were assessed on Hematoxylin/Eosin staining according to the recommendations of the International Immuno-Oncology Biomarker Working Group on Breast Cancer (10,11).

R Model marginal effect analysis

```
library(survival)
library(survminer)
library(sjPlot)
library(sjmisc)
library(ggplot2)

> COX_interaction_plot <-
coxph(Surv(Ipsilateral_recurrence_time_10y,Ipsilateral_Cens==1)~PDGFRb_score_Av
erage*RT, data=DCIS)
> plot_model(COX_interaction_plot, type = "int", colors = c("black", "red"),
grid = F) + theme_classic()
```

References for Supplementary Material and Methods

1. Strell C, Paulsson J, Jin S-B, Tobin NP, Mezheyeuski A, Roswall P, et al. Impact of Epithelial-Stromal Interactions on Peritumoral Fibroblasts in Ductal Carcinoma in Situ. J Natl Cancer Inst. 2019;

2. Paulsson J, Sjöblom T, Micke P, Pontén F, Landberg G, Heldin C-H, et al. Prognostic significance of stromal platelet-derived growth factor beta-receptor expression in human breast cancer. Am J Pathol. 2009;175:334–41.

3. Paulsson J, Rydén L, Strell C, Frings O, Tobin NP, Fornander T, et al. High expression of stromal PDGFRβ is associated with reduced benefit of tamoxifen in breast cancer. J Pathol Clin Res. 2017;3:38–43.

4. Emdin SO, Granstrand B, Ringberg A, Sandelin K, Arnesson L-G, Nordgren H, et al. SweDCIS: Radiotherapy after sector resection for ductal carcinoma in situ of the breast. Results of a randomised trial in a population offered mammography screening. Acta Oncol Stockh Swed. 2006;45:536–43.

5. Ringberg A, Nordgren H, Thorstensson S, Idvall I, Garmo H, Granstrand B, et al. Histopathological risk factors for ipsilateral breast events after breast conserving treatment for ductal carcinoma in situ of the breast--results from the Swedish randomised trial. Eur J Cancer Oxf Engl 1990. 2007;43:291–8.

6. Holmberg L, Wong YNS, Tabár L, Ringberg A, Karlsson P, Arnesson L-G, et al. Mammography casting-type calcification and risk of local recurrence in DCIS: analyses from a randomised study. Br J Cancer. 2013;108:812–9.

7. Holmberg L, Garmo H, Granstrand B, Ringberg A, Arnesson L-G, Sandelin K, et al. Absolute risk reductions for local recurrence after postoperative radiotherapy after sector resection for ductal carcinoma in situ of the breast. J Clin Oncol Off J Am Soc Clin Oncol. 2008;26:1247–52.

8. Wärnberg F, Garmo H, Emdin S, Hedberg V, Adwall L, Sandelin K, et al. Effect of radiotherapy after breast-conserving surgery for ductal carcinoma in situ: 20 years follow-up in the randomized SweDCIS Trial. J Clin Oncol Off J Am Soc Clin Oncol. 2014;32:3613–8.

9. Ranganathan P, Pramesh CS, Aggarwal R. Common pitfalls in statistical analysis: Measures of agreement. Perspect Clin Res. 2017;8:187–91.

10. Hendry S, Salgado R, Gevaert T, Russell PA, John T, Thapa B, et al. Assessing tumor infiltrating lymphocytes in solid tumors: a practical review for pathologists and proposal for a standardized method from the International Immuno-Oncology Biomarkers Working Group. Adv Anat Pathol. 2017;24:235–51.

11. Dieci MV, Radosevic-Robin N, Fineberg S, van den Eynden G, Ternes N, Penault-Llorca F, et al. Update on tumor-infiltrating lymphocytes (TILs) in breast cancer, including recommendations to assess TILs in residual disease after neoadjuvant therapy and in carcinoma in situ: A report of the International Immuno-Oncology Biomarker Working Group on Breast Cancer. Semin Cancer Biol. 2018;52:16–25.

Supplementary Tables

Supplementary table 1: Clinico-pathologic characteristics of patient-subset included in the current study in comparison to the whole SweDCIS trial cohort.			
Variable	Subset included in current study	SweDCIS all patients	p-value*
N (%)	590	1046	
Age, years:			
<50	147 (24.9)	252 (24.1)	0.782
50-60	241 (40.8)	418 (40)	
>60	202 (34.2)	376 (35.9)	
Size, mm:			
≤15	385 (65.3)	658 (62.9)	0.379
>15	162 (27.5)	292 (27.9)	
unknown	43 (7.3)	96 (9.2)	
Focality:			
unifocal	480 (81.4)	876 (83.7)	0.466
multifocal/multicentric [a]	59 (10)	92 (8.8)	
unknown	51 (8.6)	78 (7.5)	
Margin status:			
clear	486 (82.4)	857 (81.9)	0.442
not clear	71 (12)	115 (11)	
unknown	33 (5.6)	74 (7.1)	
Laterality:			
left	289 (49)	535 (51.1)	0.410
right	301 (51)	511(48.9)	
Screening detection:			
yes	464 (78.7)	823 (78.7)	0.774
no	123 (20.8)	220 (21)	
unknown	3 (0.5)	3 (0.3)	
Diagnosis year:			
≤1995	402 (68.1)	721 (68.9)	0.740
>1995	188 (31.9)	325 (31.1)	
Radiotherapy:			
yes	307 (52)	526 (50.3)	0.504
no	283 (48)	520 (59.7)	
Endocrine therapy:			
yes	19 (3.2)	33 (3.2)	1.000
no	571 (96.8)	1013 (96.8)	
Ipsilateral breast event, No. within 10 years (% of patients):			
in situ	72 (12.2)	120 (11.5)	0.689
invasive	47 (8.0)	87 (8.3)	0.851
Contralateral breast event, No. within 20 years (% of patients):			
in situ	15 (2.5)	20 (1.9)	0.477
invasive	57 (9.7)	92 (8.8)	0.592
Others, No. (% of patients):			
distant recurrence, within 20 years	17 (2.9)	34 (3.3)	0.768
BCSD, within 20 years	25 (4.2)	40 (3.8)	0.694
Death, within 20 years	104 (17.6)	183 (17.5)	0.946

RT = radiotherapy; BCSD = breast cancer specific death

* p-values are based on Fisher's exact test from contingency table analysis and are two-sided

(a) multicentric n=1 in study cohort, n=3 in original SweDCIS

Supplementary table 2: Clinico-pathologic parameters per PDGFRb group and treatment arm.									
	Total n included in current study								
	Total (n=590)			PDGFRb^{low} (n=304)			PDGFRb^{high} (n=286)		
Variable	no RT	RT	p-value*	no RT	RT	p-value*	no RT	RT	p-value*
n (%)	283	307		149	155		134	152	
Age, years:									
<50	68 (24)	79 (25.7)	0.827	32 (21.5)	34 (21.9)	0.565	36 (26.9)	45 (29.6)	0.771
50-60	119 (42)	122 (39.8)		64 (43)	58 (37.5)		55 (41)	64 (42.1)	
>60	96 (34)	106 (34.5)		53 (35.5)	63 (40.6)		43 (32.1)	43 (28.3)	
Size [a], mm:									
≤15	192 (73)	193 (68)	0.223	97 (71.9)	96 (65.3)	0.251	95 (74.2)	97 (70.8)	0.583
>15	71 (27)	91 (32)		38 (28.1)	51 (34.7)		33 (25.8)	40 (29.2)	
Nuclear grade:									
1	82 (29)	94 (30.6)	0.914	46 (30.9)	53 (34.2)	0.824	36 (20.9)	41 (27)	1.000
2	93 (32.9)	99 (32.2)		49 (32.9)	49 (31.6)		44 (32.8)	50 (32.9)	
3	108 (38.1)	114 (37.2)		54 (36.2)	53 (34.2)		54 (40.3)	61 (40.1)	
KI67 [b], % :									
< 20	191 (67.7)	208 (68.4)	0.860	106 (71.1)	108 (70.1)	0.900	85 (63.9)	100 (66.7)	0.707
≥ 20	91 (32.3)	96 (31.6)		43 (28.9)	46 (29.9)		48 (36.1)	50 (33.3)	
TILs, % :									
≤1%	73 (25.8)	71 (23.1)	0.678	39 (26.2)	37 (23.9)	0.586	34 (25.4)	34 (25.4)	0.256
>1-10%	151 (53.4)	174 (56.7)		87 (58.4)	87 (56.1)		64 (47.8)	87 (57.2)	
>10-100%	59 (20.8)	62 (20.2)		23 (15.4)	31 (20)		36 (26.8)	31 (20.4)	
Focality [c]:									
unifocal	230 (88.5)	250 (89.6)	0.681	119 (88.1)	125 (89.3)	0.850	111 (88.8)	125 (89.9)	0.842
multifocal/-centric[d]	30 (11.5)	29 (10.4)		16 (11.9)	15 (10.7)		14 (11.2)	14 (10.1)	
Margin status [e]:									
clear	233 (86.6)	253 (87.8)	0.704	122 (86.5)	131 (91.6)	0.187	111 (86.7)	122 (84.1)	0.609
not clear	36 (13.4)	35 (12.2)		19 (13.5)	12 (8.4)		17 (13.3)	23 (15.9)	
Laterality:									
left	132 (46.6)	157 (51.1)	0.285	67 (45)	79 (51)	0.304	65 (48.5)	78 (51.3)	0.722
right	151 (53.4)	150 (48.9)		82 (55)	76 (49)		69 (51.5)	74 (48.7)	
Screen detected [f]:									
screen	223 (79.4)	241 (78.8)	0.919	120 (80.5)	124 (80)	1.000	103 (78)	117 (77.5)	1.000
clinical	58 (20.6)	65 (21.2)		29 (19.5)	31 (20)		29 (22)	34 (22.5)	
Diagnosis year:									
≤1995	194 (68.6)	208 (67.8)	0.860	104 (69.8)	107 (69)	0.902	90 (67.2)	101 (66.4)	1.000
>1995	89 (31.4)	99 (32.2)		45 (30.2)	48 (31)		44 (32.8)	51 (33.6)	
Endocrine therapy:									
yes	8 (2.8)	11 (3.6)	0.648	3 (2)	5 (3.2)	0.723	5 (3.7)	6 (3.9)	1.000
no	275 (97.2)	296 (96.4)		146 (98)	150 (96.8)		129 (96.3)	146 (96.1)	
Ipsilateral breast event, No. within 10 years (% of patients):									
in situ	48 (17)	24 (7.8)	**<0.001**	28 (18.8)	7 (4.5)	**<0.001**	20 (14.9)	17 (11.2)	0.380
invasive	26 (9.2)	21 (6.8)	0.361	13 (8.7)	4 (2.5)	**0.024**	13 (9.7)	17 (11.2)	0.704

RT = radiotherapy; TILs = tumor infiltrating lymphocytes

* p-values are based on Fisher's exact test from contingency table analysis and are two-sided; (a) data missing n=43; (b) data missing of 4 patients; (c) data missing of 51 patients; (d) multicentric n=1; (e) data missing of 33 patients; (f) data missing of 3 patients

Supplementary table 3: Correlation between clinico-pathologic parameters and stromal PDGFRb status (total n=590)			
Variable	PDGFRb^{low}	PDGFRb^{high}	p-value*
N (%)	304	286	
Age:			
<50	66 (21.7)	81 (28.3)	0.066
50-60	122 (40.1)	119 (41.6)	
>60	116 (38.2)	86 (30.1)	
Size [a], mm:			
≤15	193 (68.4)	192 (72.5)	0.349
>15	89 (31.6)	73 (27.5)	
Nuclear grade:			
1	99 (32.6)	77 (26.9)	0.275
2	98 (32.2)	94 (32.9)	
3	107 (35.2)	115 (40.2)	
KI67 [b], %:			
< 20	214 (70.6)	185 (65.4)	0.184
≥ 20	89 (29.4)	98 (34.6)	
TILs, %:			
≤ 1%	76 (25)	68 (28.8)	0.235
> 1 - 10%	174 (57.2)	151 (52.8)	
> 10 - 100%	54 (17.8)	67 (23.4)	
Focality [c]:			
unifocal	244 (88.7)	236 (89.4)	0.890
multifocal/multicentric [d]	31 (11.3)	28 (10.6)	
Margin status [e]:			
clear	252 (89.1)	233 (85.3)	0.205
not clear	31 (10.9)	40 (14.7)	
Laterality:			
left	146 (48)	143 (50)	0.680
right	158 (52)	143 (50)	
Screen detected [f]:			
screen	244 (80.3)	220 (77.7)	0.478
clinical	60 (19.7)	63 (22.3)	
Diagnosis year:			
≤1995	211 (69.4)	191 (66.8)	0.536
>1995	93 (30.6)	95 (33.2)	
Radiotherapy:			
yes	155 (51)	152 (53.1)	0.621
no	149 (49)	134 (46.9)	
Endocrine therapy:			
yes	8 (2.6)	11 (3.8)	0.487
no	296 (97.4)	275 (96.2)	

TILs = tumor infiltrating lymphocytes
* p-values are based on Fisher's exact test from contingency table analysis;
(a) data missing n=43; (b) data missing of 4 patients; (c) data missing of 51 patients; (d) multicentric n=1; (e) data missing of 33 patients; (f) data missing of 3 patients

Supplementary table 4: Prognostic effect of PDGFRb for secondary endpoints at 20 years post BCS.				
	No RT		**RT**	
endpoint	**HR (95% CI), UVA** PDGFRb^{low} vs. PDGFRb^{high}	**p-value**	**HR (95% CI), UVA** PDGFRb^{low} vs. PDGFRb^{high}	**p-value**
distant recurrence	0.46 (0.09-2.37)	0.353	1.53 (0.43-5.41)	0.512
BCSD	0.66 (0.19-2.26)	0.510	1.80 (0.60-5.36)	0.294
OS	1.25 (0.77-2.02)	0.366	0.79 (0.48-1.30)	0.360
CBE	0.92 (0.43-1.96)	0.823	0.82 (0.44-1.50)	0.511

RT = radiotherapy; BCS = breast conserving surgery; BCSD = breast cancer specific death; OS = overall survival; CBE = contralateral breast event; HR = cause-specific hazard ratio; CI = 95% confidence interval; UVA = univariable analysis

Supplementary table 5: Hazard ration with 95% CI within PDGFRb-defined patient groups over time after BCS for DCIS.			
	PDGFRb^{low}	**PDGFRb^{high}**	**Interaction**
Time [years]	HR (95% CI), UVA; p-value No RT vs. RT	HR (95% CI), UVA; p-value No RT vs. RT	p-value
0-5	0.18 (0.07-0.43); <0.001	0.51 (0.27-0.95); 0.034	**0.025**
5-10	0.37 (0.13-1.06); 0.064	2.01 (0.83-4.84); 0.121	**0.006**
10-20	0.87 (0.30-2.47); 0.787	0.61 (0.14-2.74); 0.521	0.366
0-20	0.32 (0.19-0.56); <0.001	0.80 (0.51-1.27); 0.354	0.012

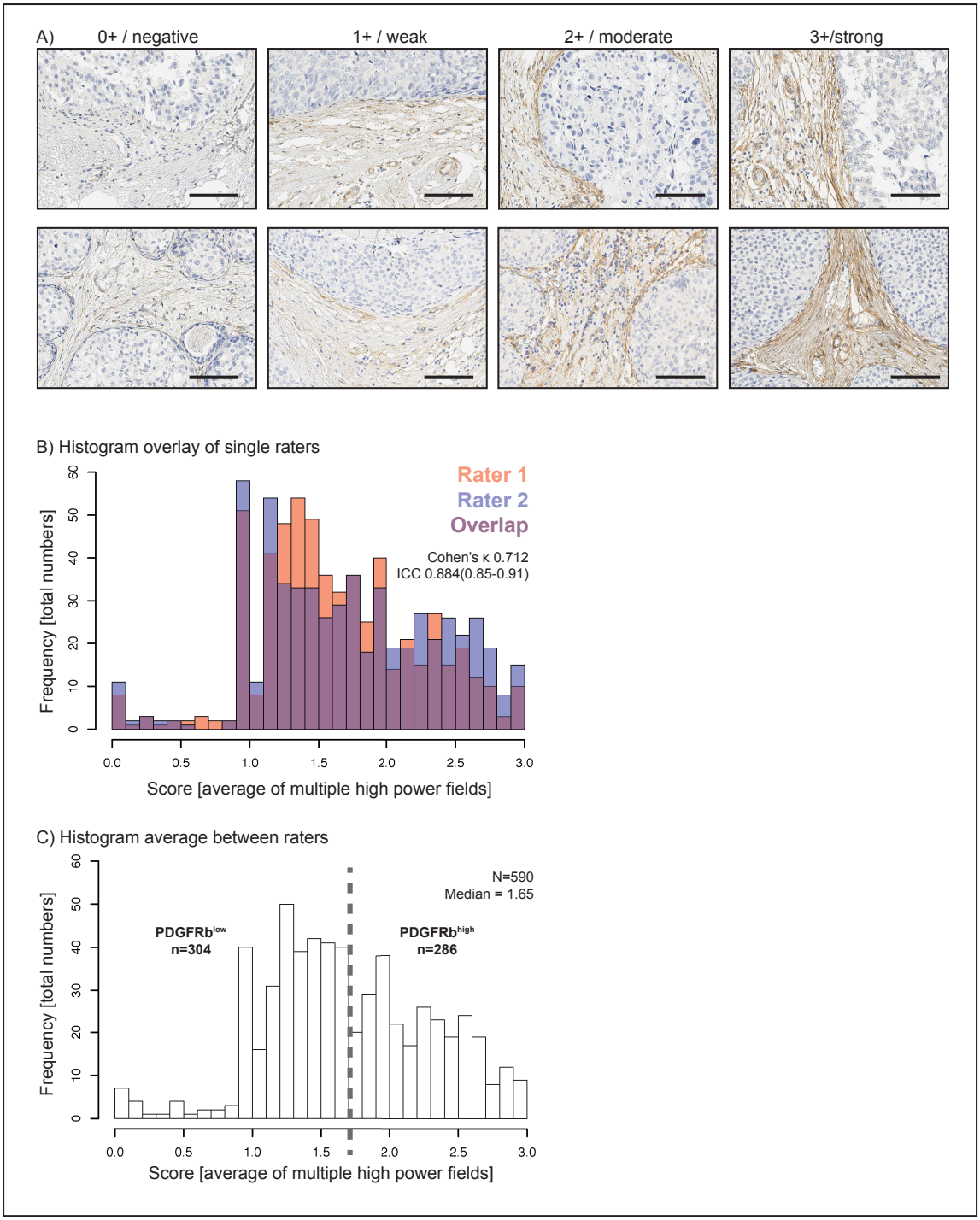
RT = radiotherapy; BCS = breast conserving surgery; HR = hazard ratio; CI = 95% confidence interval; UVA = univariable analysis

Supplementary table 6: Predictive effect of PDGFRb on benefit from RT for secondary endpoints at 20 years post BCS.				
	PDGFRb^{low}		**PDGFRb^{high}**	
endpoint	**HR (95% CI), UVA** no RT vs. RT	**p-value**	**HR (95% CI), UVA** no RT vs. RT	**p-value**
distant recurrence	0.80 (0.22-3.00)	0.742	2.62 (0.53-13.00)	0.238
BCSD	0.72 (0.23-2.28)	0.580	1.94 (0.60-6.31)	0.269
OS	1.12 (0.69-1.80)	0.652	0.71 (0.43-1.17)	0.178
CBE	1.58 (0.82-3.02)	0.172	1.40 (0.68-2.88)	0.362

RT = radiotherapy; BCS = breast conserving surgery; BCSD = breast cancer specific death; OS = overall survival; CBE = contralateral breast event; HR = cause-specific hazard ratio; CI = 95% confidence interval; UVA = univariable analysis

Supplementary Figures

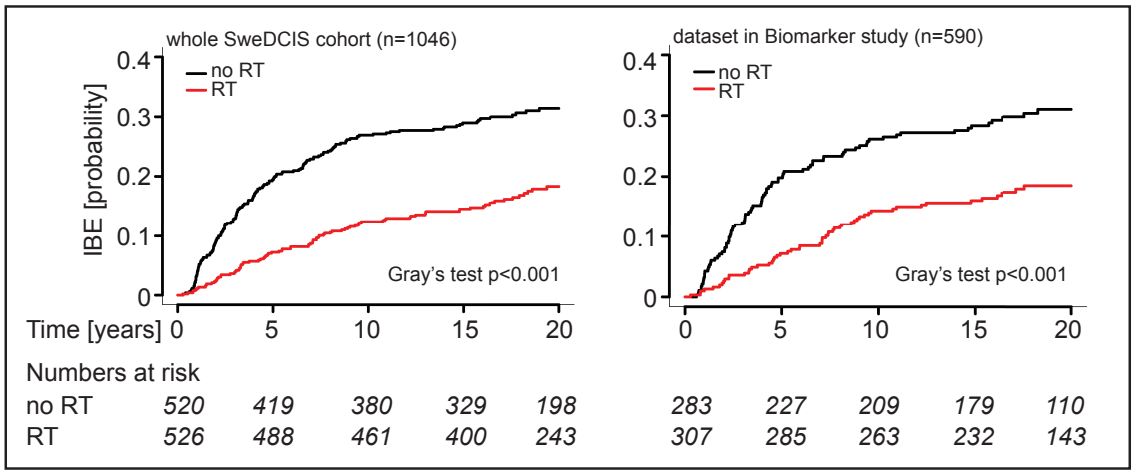
Supplementary Figure 1



Supplementary Figure 1: *PDGFRb marker evaluation*

(A) Breast DCIS: example pictures of PDGFRb expression detected by immunohistochemistry. High-power fields were scored for average intensity of the PDGFRb positive stroma fraction from none/negative (0+), low (1+), moderate (2+) to strong (3+). The scale bar represents 100um.
(B) Histogram of average PDGFRb score distribution of each rater and their overlap. Ten high power fields were scored per rater (see Materials and Methods for details) and thereof the average PDGFRb score had been calculated. Equally weighted Cohen's kappa coefficient was calculated to evaluate the inter-rater reliability. The Interclass Correlation Coefficient (ICC) with 95% confidence interval is also indicated.
(C) Histogram of final score distribution after averaging the evaluations of the two raters. The PDGFRb "low" and PDGFRb "high" group were defined by splitting the data at the median (indicated by dashed line).

Supplementary Figure 2

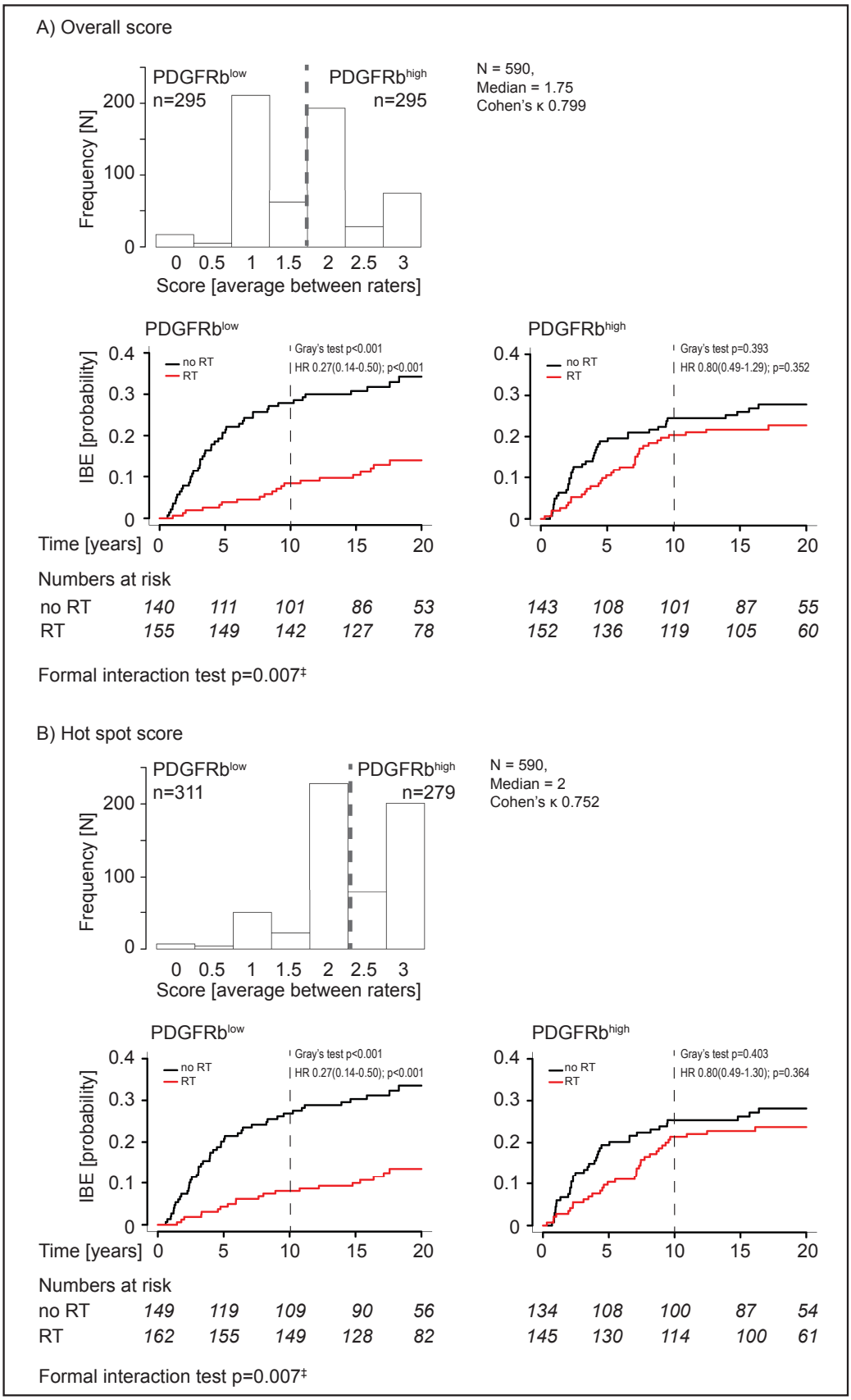


Supplementary Figure 2: *RT effect in whole SweDCIS cohort and the biomarker study cohort over 20 years post breast conserving surgery*

Comparison for cumulative incidence of ipsilateral breast events (in situ and invasive) in dependency of adjuvant radiotherapy treatment between the whole SweDCIS cohort (left panel) and the current biomarker study cohort (right panel). Gray's test was applied for comparison of the cumulative incidence functions.

RT = radiotherapy; IBE = ipsilateral breast event

Supplementary figure 3



Supplementary Figure 3: *RT effect in PDGFRb-defined patient subsets using different scoring approaches*

Alternative scoring methods: (A) "Overall integrated" PDGFRb score and (B) the "hot spot" PDGFRb score (see materials and methods for detail). Histogram of score distributions with cut-off are presented (left panels). Equally weighted Cohen's kappa coefficient was calculated to evaluate the inter-rater reliability. Graphs show cumulative incidence of ipsilateral breast events (in situ and invasive) in dependency of radiotherapy. Gray's test was applied for comparison of the cumulative incidence functions over 10 years. Hazard ratios (no-RT-group as reference) are indicated with 95% confidence interval (in parenthesis) and p-values are based on Wald test.

HR = Hazard Ratio; IBE = ipsilateral breast event
‡ NOTE Formal interaction test is based on the continuous variable and unadjusted for other clinical parameters.