Supplementary material for Tramm T, et al. Relationship between the prognostic and predictive value of the intrinsic subtypes and a validated gene profile predictive of loco-regional control and benefit from post-mastectomy radiotherapy in patients with high risk breast cancer. Acta Oncol 2014;53:1337–46.

Supplementary Table I. Antibody and FISH probe information.

Primary antibody/ FISH probe	Clone	Manufacturer	Dilution	Detection system	Pre-treatment
ER	6F11	Ventana Medical Systems, AZ, USA	Pre-diluted	Dako EnVision™	Incubation for 30 min at 20°C
PR	636	DakoCytomation, Glostrup, Denmark	1:2400	Dako EnVision™	TEG buffer, pH 9.0 Incubation over night at 4°C
HER2	HercepTest™Kit	DakoCytomation, Glostrup, Denmark	–	–	Incubation for 30 min at 20°C
EGFR	K1492	DakoCytomation, Glostrup, Denmark	Pre-diluted	Dako EnVision™	Proteinase-K Incubation for 30 min at 20°C
Ki-67	MIB-1 (M7240)	DakoCytomation, Glostrup, Denmark	1:800	Dako EnVision™	TEG buffer, pH 7.6 Incubation over night at 4°C
CK5/6	M7237	DakoCytomation, Glostrup, Denmark	1:50	Ventana Ultraview™	CC1 buffer, pH 8.5 HIER
HER2 FISH	HER2 FISH pharmDX™ Kit	DakoCytomation, Glostrup, Denmark	–	–	Incubation overnight at 45°C

Staining system: Ventana BenchMarkXT

Supplementary Table II. Positive agreement with 95% confidence intervals in parentheses for subtypes with the same nomenclature.

		CC N = 190				
		LumA	LumB	HER2	Basal	Normal
PAM50 N = 191	Luminal A	51 % (39–63)	-	-	-	-
	Luminal B	-	43% (23–66)	-	-	-
	HER2	-	-	83% (67–94)	-	-
	Basal	-	-	-	93% (77–99)	-
	Normal	-	-	-	-	50% (31–69)
IHC3 N = 146	"Luminal A"	94% (83-99)	-	-	-	-
	"Luminal B"	-	32% (13–57)	-	-	-
	"HER2"	-	-	48% (29–68)	-	-
	"Basal"	-	-	-	68% (46–85)	-
IHC6 N = 137	"Luminal A"	62% (47–76)	-	-	-	-
	"Luminal B"	-	33% (13–59)	-	-	-
	"Luminal HER2"	-	-	-	-	-
	"HER2"	-	-	50% (30–70)	-	-
	"Core basal"	-	-	-	-	-
	"TNP-nonbasal"	-	-	-	-	-

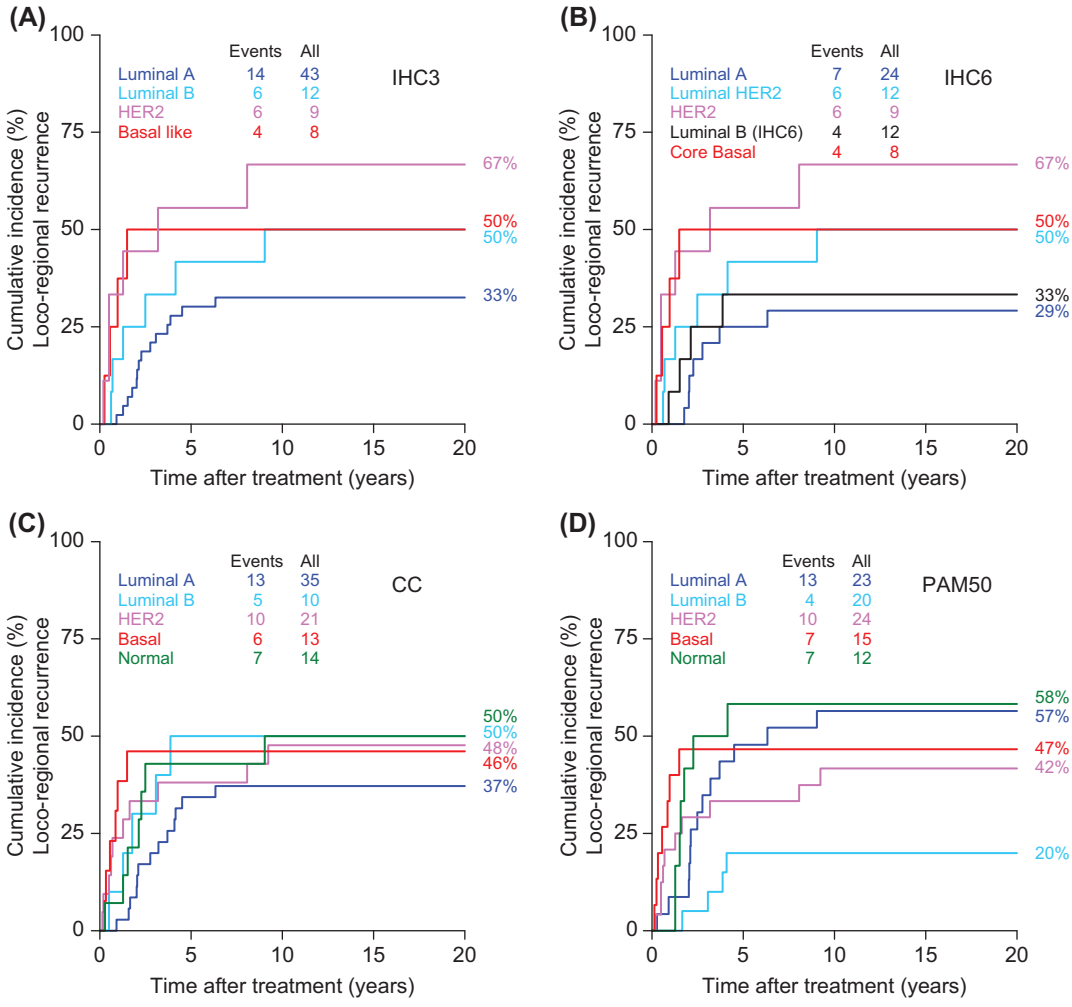
		PAM50 N = 191				
		LumA	LumB	HER2	Basal	Normal
IHC3 N = 146	"Luminal A"	86% (71–95)	-	-	-	-
	"Luminal B"	-	7% (1–23%)	-	-	-
	"HER2"	-	-	35% (20–53)	-	-
	"Basal"	-	-	-	54% (34–73)	-
IHC6 N = 137	"Luminal A"	73% (54–87)	-	-	-	-
	"Luminal B"	-	50% (30–70)	-	-	-
	"Luminal HER2"	-	-	-	-	-
	"HER2"	-	-	36% (21–54)	-	-
	"Core basal"	-	-	-	-	-
	"TNP-nonbasal"	-	-	-	-	-

		IHC3 N = 146			
		"LumA"	"LumB"	"HER2"	"Basal"
IHC6 N = 137	"Luminal A"	65% (53–75)	-	-	-
	"Luminal B"	-	0%	-	-
	"Luminal HER2"	-	-	-	-
	"HER2"	-	-	100%	-
	"Core basal"	-	-	-	-
	"TNP-nonbasal"	-	-	-	-

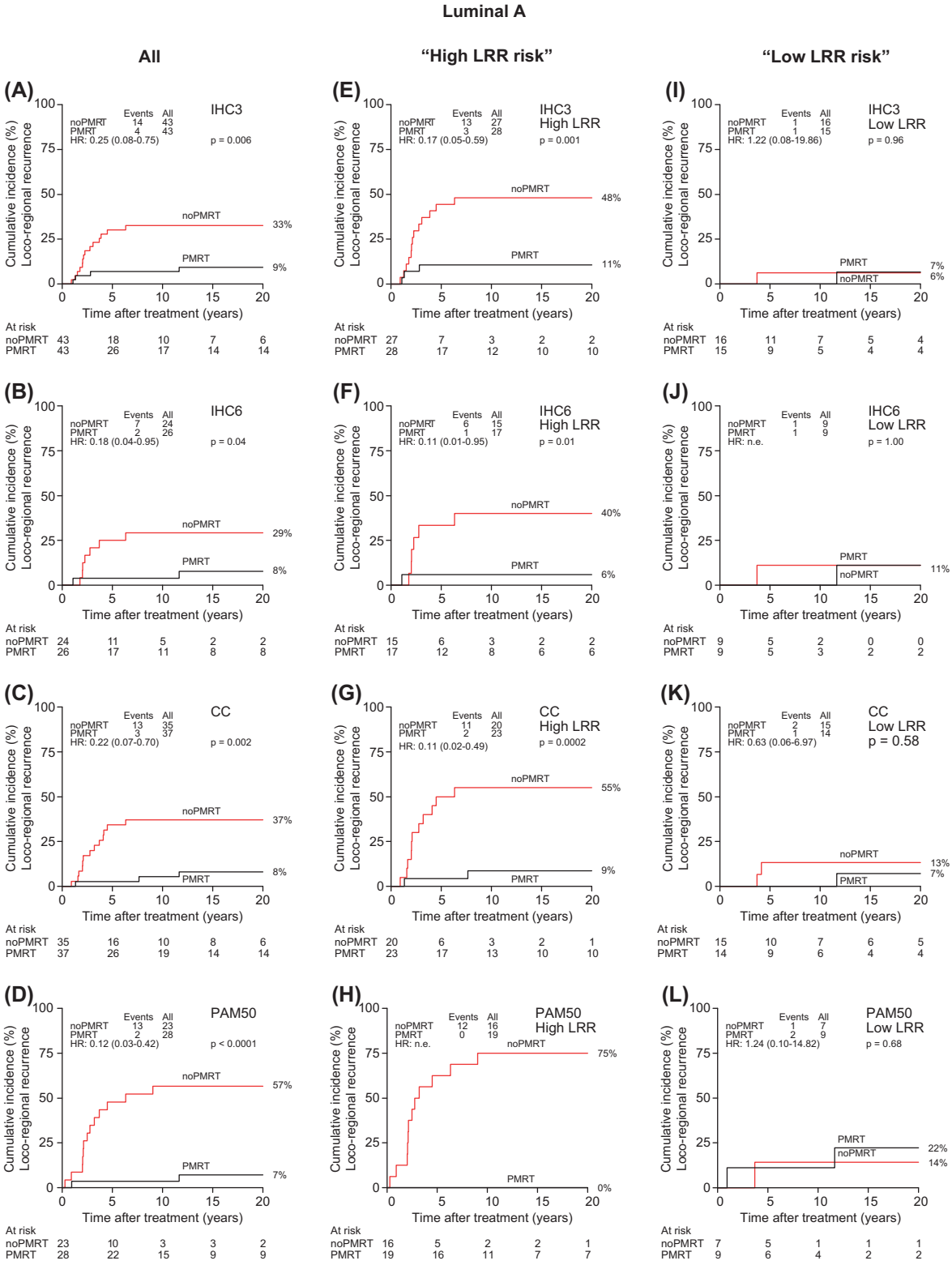
Supplementary Table III. Distribution of the two risk groups as determined by the DBCG-RT gene profile in comparison to the intrinsic subtypes. The table also shows the distribution of loco-regional recurrences (LRR) among patients treated with or without post-mastectomy radiotherapy (PMRT) among the subtypes as determined by different methods.

			"Low LRR risk"				"High LRR risk"				
			No PMRT		PMRT		No PMRT		PMRT		
Intrinsic subtypes determined by differentmethods	All patients (n = 191)		No LRR	+ LRR	No LRR	+ LRR	No LRR	+ LRR	No LRR	+ LRR	
	n	*%*	*n (%)*	*n (%)*	*n (%)*	*n (%)*	*n (%)*	*n (%)*	*n (%)*	*n (%)*	p*
FFT (n = 191)			*n = 23*	*n = 2*	*n = 21*	*n = 2*	*n = 30*	*n = 39*	*n = 64*	*n = 10*	
CC											0.0001
Luminal A	72	*38*	13 *(87%)*	*2 (13%)*	13 *(93%)*	1 *(7%)*	9 *(45%)*	11 *(55%)*	20 *(87%)*	3 *(13%)*	
Luminal B	23	*12*	2	0	5	0	3 *(27%)*	5 *(63%)*	7 *(87%)*	1 *(13%)*	
HER2	36	*19*	5	0	1	0	6 *(27%)*	10 *(63%)*	11 *(79%)*	3 *(21%)*	
Basal	29	*15*	0	0	0	0	7 *(54%)*	6 *(46%)*	13 *(81%)*	3 *(19%)*	
Normal	30	*16*	3	0	2 *(67%)*	1 *(33%)*	4 *(36%)*	7 *(64%)*	13	0	
Unknown	1	*1*	0	0	0	0	1	0	0	0	
PAM50											0.007
Luminal A	51	*27*	6 *(86%)*	*1 (14%)*	7 *(78%)*	2 *(22%)*	4 *(25%)*	12 *(75%)*	18 *(95%)*	1 *(5%)*	
Luminal B	37	*19*	8	*0*	8	0	8 *(67%)*	4 *(33%)*	6 *(67%)*	3 *(33%)*	
HER2	45	*24*	6	*0*	3	0	8 *(44%)*	10 *(56%)*	16 *(89%)*	2 *(11%)*	
Basal	36	*19*	1	*0*	2	0	7 *(50%)*	7 *(50%)*	15 *(79%)*	4 *(21%)*	
Normal	22	*12*	2 *(67%)*	*1 (33%)*	1	0	3 *(33%)*	6 *(67%)*	9	0	
FFPE (n = 146)			*n = 17*	*n = 2*	*n = 18*	*n = 2*	*n = 25*	*n = 28*	*n = 46*	*n = 8*	
IHC3											0.01
"Luminal A"	86	*59*	15 *(94%)*	1 *(6%)*	14 *(93%)*	1 *(7%)*	14 *(52%)*	13 *(48%)*	25 *(89%)*	3 *(11%)*	
"Luminal B"	25	*17*	1 *(50%)*	1 *(50%)*	3	0	5 *(50%)*	5 *(50%)*	8 *(80%)*	2 *(20%)*	
"HER2"	16	*11*	1	0	0	1	2 *(25%)*	6 *(75%)*	4 *(67%)*	2 *(33%)*	
"Basal"	19	*13*	0	0	1	0	4 *(50%)*	4 *(50%)*	9 *(90%)*	1 *(10%)*	
IHC6											0.09
"Luminal A"	50	*34*	8 *(89%)*	1 *(11%)*	8 *(89%)*	1 *(11%)*	9 *(60%)*	6 *(40%)*	16 *(94%)*	1 *(6%)*	
"Luminal B"	27	*19*	3	0	5	0	5 *(56%)*	4 *(44%)*	8 *(80%)*	2 *(20%)*	
"Luminal HER2"	25	*17*	1*(50%)*	1 *(50%)*	3	0	5 *(50%)*	5 *(50%)*	8 *(80%)*	2 *(20%)*	
"HER2"	16	*11*	1	0	0	1	2 *(25%)*	6 *(75%)*	4 *(67%)*	2 *(33%)*	
"Core basal"	16	*11*	0	0	1	0	4 *(50%)*	4 *(50%)*	6 *(86%)*	1 *(14%)*	
"TNP-non-basal"	3	*2*	0	0	0	0	0	0	3	0	
Unknown	9	*6*	4	0	1	0	0	3	1	0	

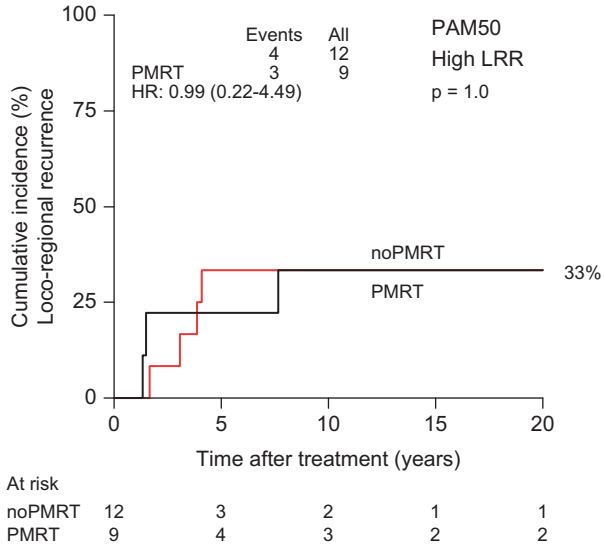
*Fischer Exact test for comparison between distribution of "High LRR risk" and "Low LRR risk" for each subtype.



Supplementary Figure 1. Plots of cumulative incidence proportion of locoregional recurrence showing slightly different clinical outcome in the 94/191 non-irradiated DBCG2bc patients (A) IHC3 (B) IHC6 (C) CC; intrinsic subtypes as determined by the original centroid correlation based method and (D) PAM50.



Supplementary Figure 2. Predictive value of PMRT for patients with tumors exhibiting Luminal A features determined by various approaches: (A) IHC3, (B) IHC6, (C) CC and (D) PAM50. Patients with Luminal A tumors categorized as "High LRR risk" according to the DBCG-RT gene profile can be seen to gain benefit from PMRT (E-H), whereas patients with a "low LRR risk" profile shows no additional benefit from PMRT despite of Luminal A features (I-L).



Supplementary Figure 3. PAM50 determined Luminal B subtype showing a fairly high risk of LRR but without benefit from PMRT in the limited number of patients in the "High LRR risk" profile group.