Supplementary Online Content

Goddard ET, Bassale S, Schedin T, et al. Association between postpartum breast cancer diagnosis and metastasis and the clinical features underlying risk. *JAMA Netw Open*. 2019;2(1):e186997. doi:10.1001/jamanetworkopen.2018.6997

This supplementary material has been provided by the authors to give readers additional information about their work.

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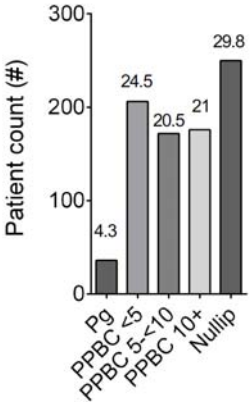

eFigure 1. Postpartum breast cancers diagnosed within 10 years of a completed pregnancy account for 45% of all young women's breast cancers. Patient counts by parity status, including the subset of patients diagnosed while pregnant (Pg). Patients diagnosed within 10 years of a pregnancy account for 45% of all patients in this cohort and pregnant patients account for 4.3%. Pg, n=36; PPBC<5, n=206; PPBC 5-<10, n=172; PPBC 10+, n=176; Nullip, n=250.

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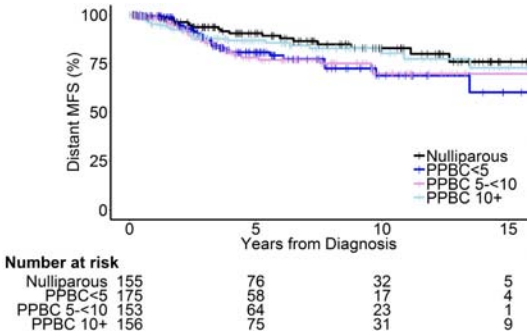

eFigure 2. Exclusion of nulliparous patients with incomplete pregnancies does not impact distant metastasis-free survival. Distant metastasis-free survival (MFS) in the Colorado Young Women's Breast Cancer Cohort in which nulliparous patients with incomplete pregnancies were excluded from analysis (Log Rank Test, P=0.02). Nullip, n=155; PPBC<5, n=175; PPBC 5-<10, n=153; PPBC 10+, n=156; Nullip with incomplete pregnancies excluded from analysis, n=62.

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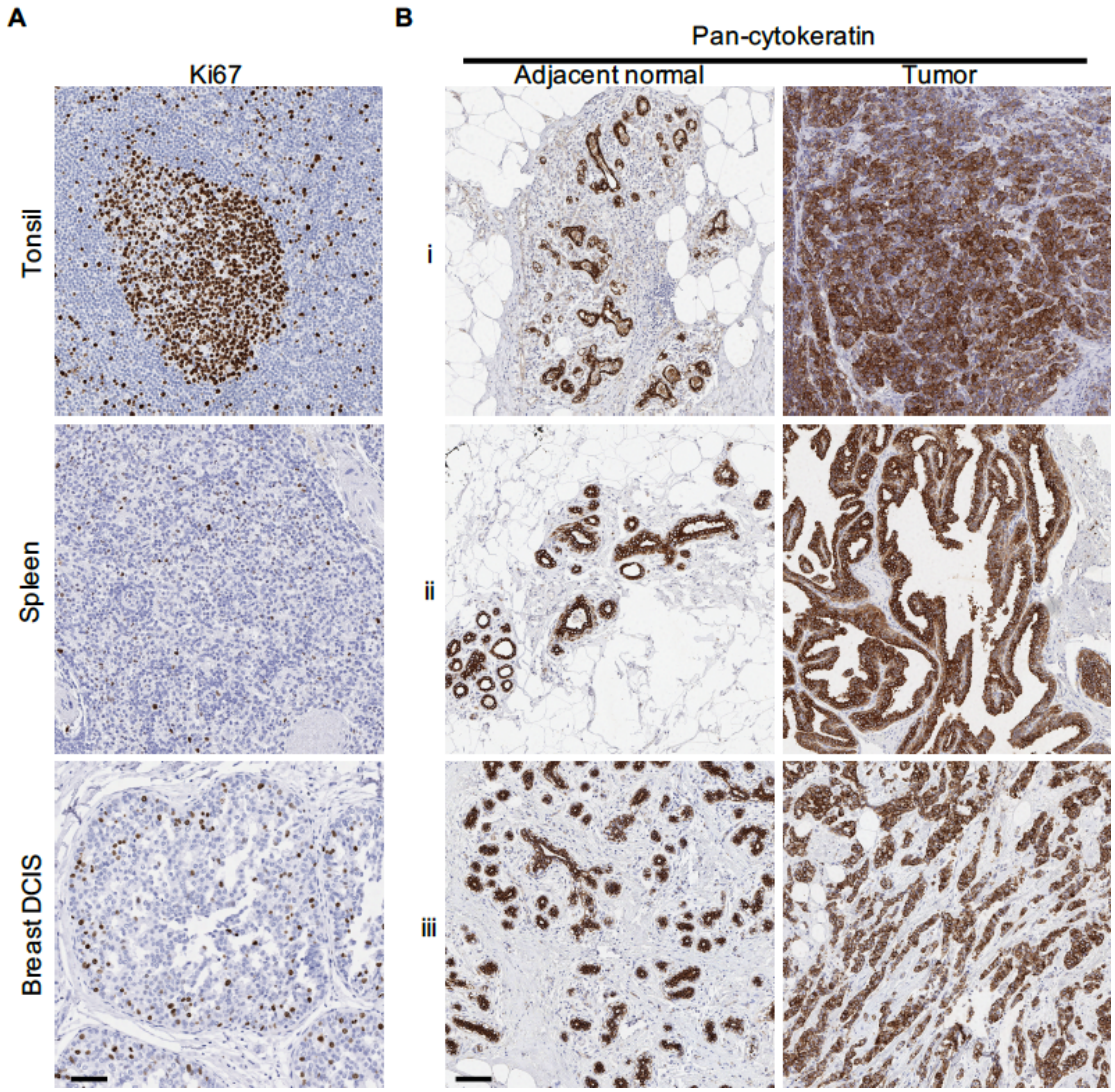

eFigure 3. Patient tissue immunohistochemistry staining controls. (A) Representative Ki67 staining in human tonsil (top), spleen (middle), and breast ductal carcinoma in situ (DCIS; bottom) served as positive controls on staining runs (scale bar = 100 μm) **(B)** Pan-cytokeratin staining to confirm antigenicity in three (i-iii) patient breast tumor samples that had low or negative antigen staining levels for numerous stains including Ki67. Representative pan-cytokeratin staining in adjacent normal breast tissue (left) and tumor (right) (scale bar = 300 μm).

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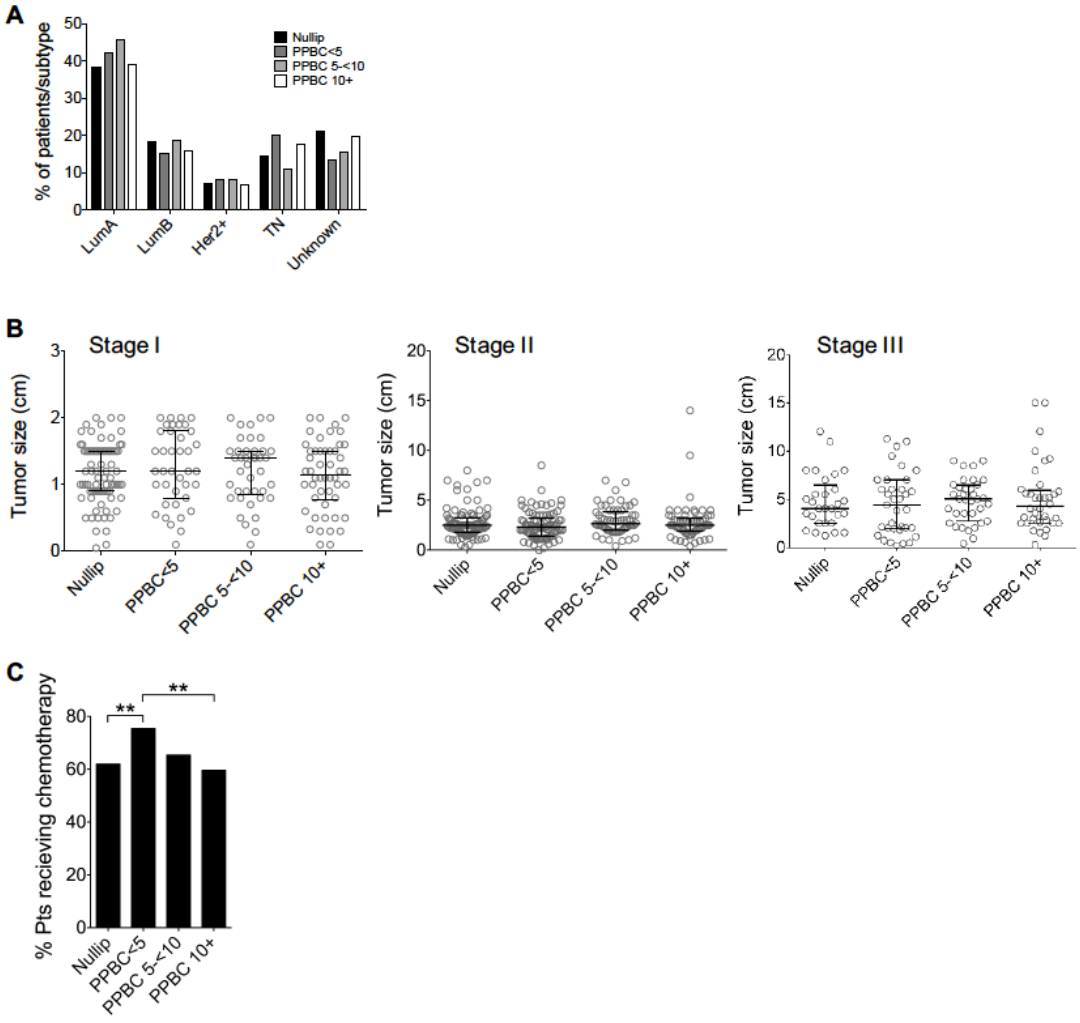

eFigure 4. Biologic subtype and tumor size are not altered by parity status of young women's breast cancer patients. (A) Biologic subtype at diagnosis in Stage I-III patients, categorized by parity group (two-way ANOVA, Tukey's multiple comparisons test). Luminal A (LumA): Nullip, n=85; PPBC<5, n=75; PPBC 5-<10, n=70; PPBC 10+, n=61. Luminal B (LumB): Nullip, n=41; PPBC<5, n=27; PPBC 5-<10, n=29; PPBC 10+, n=25. Her2+: Nullip, n=16; PPBC<5, n=15; PPBC 5-<10, n=13; PPBC 10+, n=11. TN (triple negative): Nullip, n=32; PPBC<5, n=36; PPBC 5-<10, n=17; PPBC 10+, n=28. Unknown: Nullip, n=47; PPBC<5, n=24; PPBC 5-<10, n=24; PPBC 10+, n=31. **(B)** Tumor size at diagnosis in stage I (left), stage II (middle), and stage III (right) patients (one-way ANOVA, Tukey's multiple comparisons test; error bars are median with interquartile range). **(C)** Percentage of Stage I-III patients treated with chemotherapy categorized by parity status (chi-squared test). Nullip: n=137 chemotherapy (chemo)+, n=40 chemo-, n=40 unknown; PPBC<5: n=134 chemo+, n=15 chemo-, n=28 unknown; PPBC 5-<10: n=100 chemo+, n=25 chemo-, n=28 unknown; PPBC 10+: n=93 chemo+, n=22 chemo-, n=41 unknown. **=p-value<0.01.

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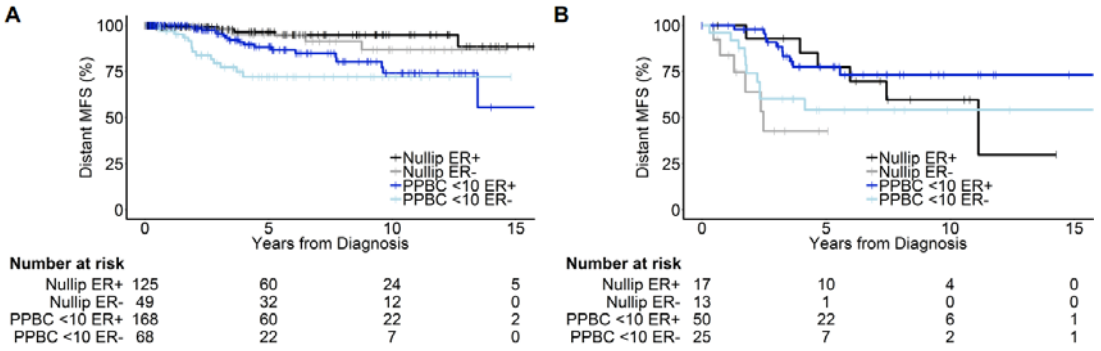

eFigure 5. Interactions between parity, estrogen receptor status, and stage determine distant metastasis-free survival in young women's breast cancer. (A) Distant metastasis-free survival (MFS) in stage I/II patients categorized by parity and estrogen receptor (ER) status. Nullip ER+, n=125; Nullip ER-, n=49; PPBC<10 ER+, n=168; PPBC<10 ER-, n=68 (Log Rank Test, P<0.0001, see eTable 2). **(B)** Distant MFS in stage III patients categorized by parity and ER status. Nullip ER+, n=17; Nullip ER-, n=13; PPBC<10 ER+, n=50; PPBC<10 ER-, n=25 (Log Rank Test, P=0.005, see eTable 2).

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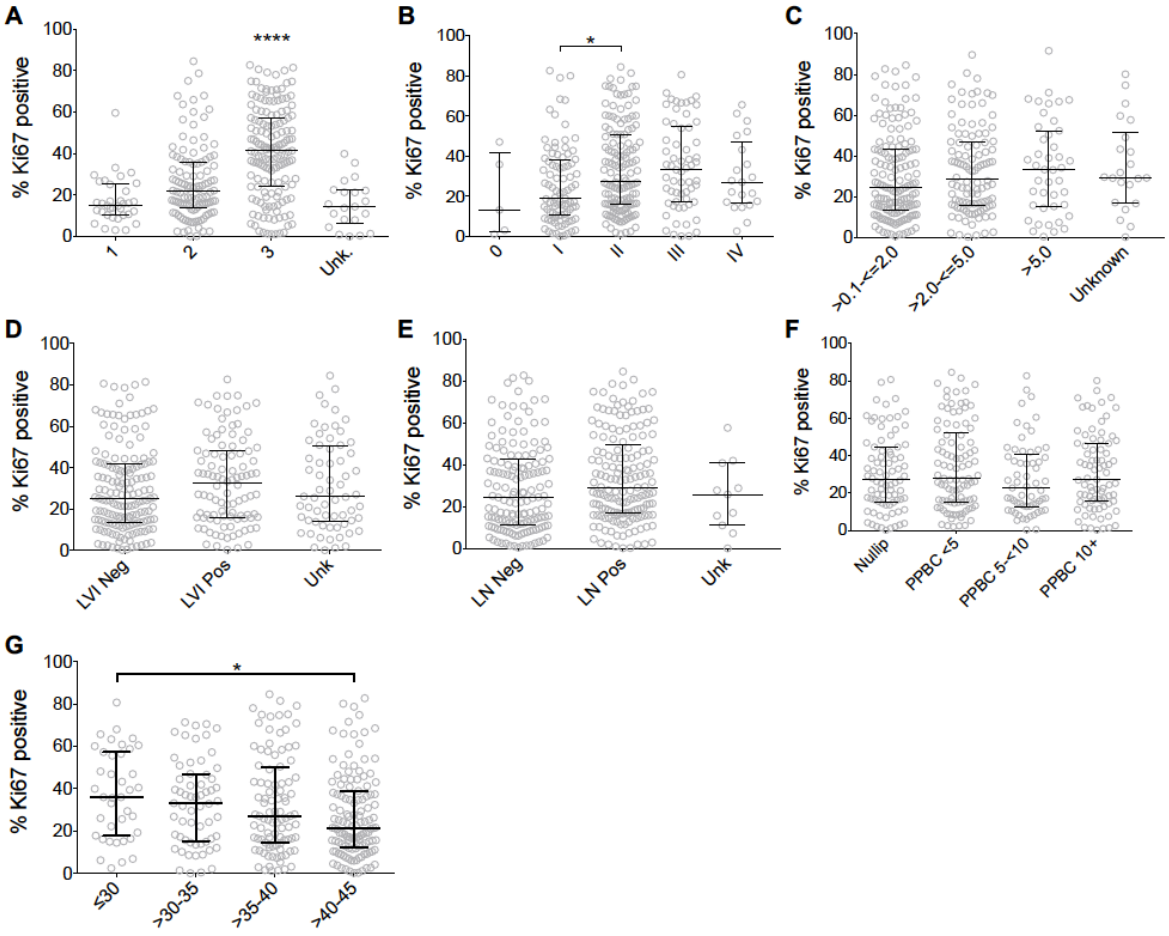

eFigure 6. Intratumoral Ki67 staining index is increased with increasing grade and stage and is reduced with increasing age at diagnosis. Intratumoral Ki67 staining indices by pathological grade **(A)**, clinical stage **(B)**, tumor size **(C)**, lymphovascular invasion (LVI) **(D)**, lymph node (LN) involvement **(E)**, parity status **(F)**, and age at diagnosis **(G)** (One-way ANOVA, Tukey's multiple comparisons test; error bars are median with interquartile range). Nulliparous (Nullip); postpartum breast cancer (PPBC); unknown (unk); positive (Pos); negative (neg). *=p-value<0.05, ****=p-value<0.0001.

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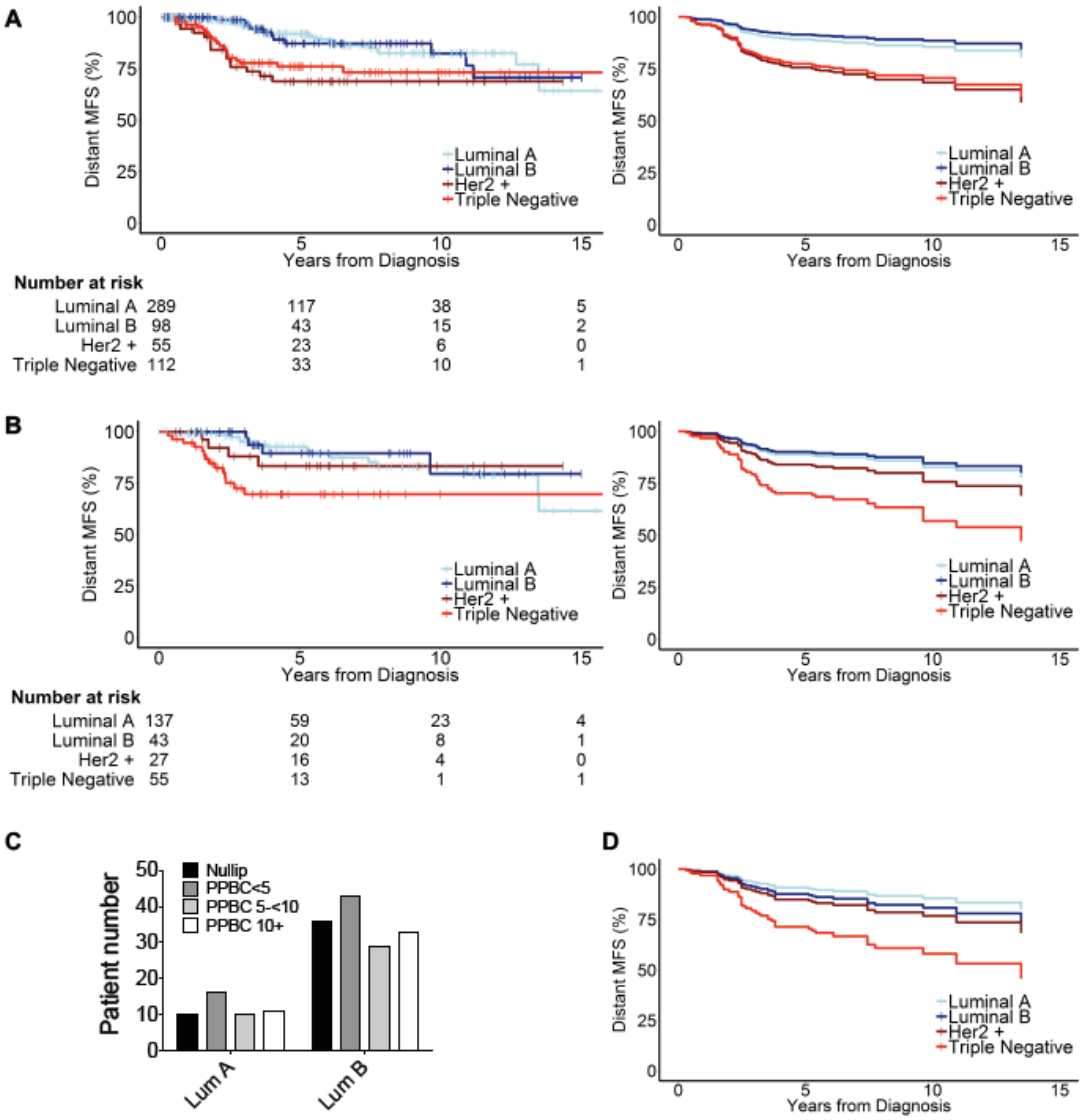

eFigure 7. Distant MFS in the Colorado Young Women's Breast Cancer Cohort by luminal subtype. (A) Distant MFS in the overall Colorado Young Women's Breast Cancer Cohort, categorized by biologic subtype using historic subtype definitions: Luminal A is defined as ER+, PR+, Her2- and luminal B is defined as ER+, PR-, Her2- or ER+, PR+/-, Her2+. Data is unadjusted (left) or adjusted for tumor size, age at diagnosis, and year of diagnosis (right). Luminal A, n=289; luminal B, n=98; Her2+, n=55; Triple negative, n=112. See eTable 4 for statistics. **(B)** Distant MFS in the Ki67 stained subset cohort, categorized by biologic subtype, using the historic Luminal A and B definitions as described in Fig. 4A-B. Data is unadjusted (left) or adjusted for tumor size, age at diagnosis, and year of diagnosis (right). Luminal A, n=137; luminal B, n=43; Her2+, n=27; Triple negative, n=55. See eTable 4 for statistics. **(C)** Number of patients classified as luminal A or luminal B using the Ki67-based definition and categorized by parity group. **(D)** Distant MFS in the Ki67 stained subset cohort, categorized by biologic subtype using the Ki67-based biologic subtype definitions: luminal A is defined as ER+, PR+, Her2-, Ki67 low; luminal B is defined as ER+, PR+/-, Her2+/-, Ki67 high. Data is adjusted for tumor size, age at diagnosis, and year of diagnosis. See eTable 4 for statistics.

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eTable 1 | University of Colorado metastasis-free survival analysis

UC Cohort Statistical Analysis, Stage I, II, II (N=701)					
	Log Rank Test P value	Univariate P value	Hazard Ratio [95% CI]	Multivariate P value*	Hazard Ratio [95% CI]
Group					
Nulliparous (n=217)		Reference	Reference	Reference	Reference
PPBC<5 (n=175)	0.02	0.009	2.13 [1.21-3.74]	0.16	1.53 [0.85-2.75]
PPBC 5-<10 (n=153)		0.006	2.23 [1.26-3.93]	0.02	1.99 [1.10-3.59]
PPBC 10+ (n=156)		0.13	1.56 [0.87-2.81]	0.30	1.40 [0.75-2.61]
UC Cohort Statistical Analysis, Stage I, II patients only (N=550)					
	Log Rank Test P value	Univariate P value	Hazard Ratio [95% CI]	Multivariate P value*	Hazard Ratio [95% CI]
Group					
Nulliparous (n=185)		Reference	Reference	Reference	Reference
PPBC<5 (n=137)	0.001	0.001	3.69 [1.65-8.25]	0.002	3.51 [1.56-7.89]
PPBC 5-<10 (n=116)		<0.001	4.06 [1.81-9.15]	<0.001	5.16 [2.26-11.82]
PPBC 10+ (n=112)		0.12	2.02 [0.84-4.89]	0.05	2.57 [1.02-6.48]
UC Cohort Statistical Analysis, Stage III patients only (N=151)					
	Log Rank Test P value	Univariate P value	Hazard Ratio [95% CI]	Multivariate P value*	Hazard Ratio [95% CI]
Group					
Nulliparous (n=32)		Reference	Reference	Reference	Reference
PPBC<5 (n=38)	0.60	0.38	0.70 [0.31-1.58]	0.14	0.49 [0.19-1.26]
PPBC 5-<10 (n=40)		0.19	0.58 [0.26-1.31]	0.30	0.65 [0.28-1.48]
PPBC 10+ (n=41)		0.36	0.69 [0.31-1.52]	0.39	0.69 [0.29-1.61]

*** Multivariable Cox Proportional Hazards regression, adjusted for biologic subtype, age at diagnosis, and year of diagnosis**
95% CI = 95% confidence interval; UC = University of Colorado

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eTable 2 | University of Colorado metastasis-free survival analysis, stratified by ER status

UC Cohort Statistical Analysis, Stage I, II, III stratified by ER status (N=515)

	Log Rank Test P value	Univariate P-value	Hazard Ratio [95% CI]	Multivariate P-value*	Hazard Ratio [95% CI]
Group					
Nulliparous, ER+ (n=142)	<0.001	Reference	Reference	Reference	Reference
Nulliparous, ER- (n=62)		0.06	1.95 [0.97-3.93]	0.20	1.60 [0.79-3.26]
PPBC<10, ER+ (n=218)		0.12	1.99 [0.84-4.68]	0.19	1.80 [0.75-4.33]
PPBC<10, ER- (n=93)		<.001	4.34 [2.12-8.89]	0.004	3.02 [1.44-6.36]

UC Cohort Statistical Analysis, Stage I, II patients only, stratified by ER status (N=410)

	Log Rank Test P value	Univariate P-value	Hazard Ratio [95% CI]	Multivariate P-value**	Hazard Ratio [95% CI]
Group					
Nulliparous, ER+ (n=125)	<0.001	Reference	Reference	Reference	Reference
Nulliparous, ER- (n=49)		0.02	3.40 [1.26-9.21]	0.008	3.84 [1.42-10.42]
PPBC<10, ER+ (n=168)		0.45	1.66 [0.45-6.2]	0.77	1.22 [0.32-4.62]
PPBC<10, ER- (n=68)		<0.001	7.07 [2.53-19.82]	<0.001	6.37 [2.26-17.96]

UC Cohort Statistical Analysis, Stage III patients only, stratified by ER status (N=105)

	Log Rank Test P value	Univariate P-value	Hazard Ratio [95% CI]	Multivariate P-value**	Hazard Ratio [95% CI]
Group					
Nulliparous, ER+ (n=17)	0.005	Reference	Reference	Reference	Reference
Nulliparous, ER- (n=13)		0.34	0.61 [0.22-1.69]	0.19	0.49 [0.17-1.43]
PPBC<10, ER+ (n=50)		0.05	3.26 [1.02-10.45]	0.29	2.04 [0.55-7.54]
PPBC<10, ER- (n=25)		0.38	1.57 [0.57-4.35]	0.78	1.17 [0.39-3.50]

*** Multivariate logistic regression, adjusted for biologic subtype, stage, age at diagnosis, and year of diagnosis**
**** Multivariate logistic regression, adjusted for biologic subtype, age at diagnosis, and year of diagnosis**
95% CI = 95% confidence interval; UC = University of Colorado; ER = estrogen receptor

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eTable 3 | Ki67 staining cohort

	Nulliparous (N=72) No. (%)	PPBC <5 years (N=86) No. (%)	PPBC 5-<10 years (N=48) No. (%)	PPBC 10+ (N=66) No. (%)	P-value
Mean age at diagnosis	37.2	34.8	39.2	41.5	<0.001[b]
Biologic subtype					
Luminal A (ER+, PR+, Her2 neu-)	30 (41.7%)	45 (52.3%)	30 (62.5%)	32 (48.5%)	
Luminal B (ER+, PR+/-, Her2 neu+)	14 (19.4%)	10 (11.6%)	8 (16.7%)	10 (15.2%)	
Her2 neu positive (ER-, PR-)	12 (16.7%)	9 (10.5%)	2 (4.2%)	4 (6.1%)	0.35[a]
Triple negative	14 (19.4%)	20 (23.3%)	7 (14.6%)	16 (24.2%)	
Luminal unknown	2 (2.8%)	2 (2.3%)	1 (2.1%)	4 (6.1%)	
Updated biologic subtype					
Luminal A (ER+, PR+/-, Ki67 <14%)	9 (12.5%)	14 (16.3%)	10 (20.83%)	11 (16.7%)	
Luminal B (ER+, PR+/-, Ki67 ≥14%)	31 (43.1%)	38 (44.2%)	27 (56.3%)	33 (50.0%)	
Her2 neu positive (ER-, PR-)	12 (16.7%)	9 (10.5%)	2 (4.2%)	4 (6.1%)	0.45[a]
Triple negative	14 (19.4%)	20 (23.3%)	7 (14.6%)	16 (24.2%)	
Luminal unknown	6 (8.3%)	5 (5.8%)	2 (4.2%)	2 (3.0%)	
Estrogen status					
ER+	46 (63.9%)	57 (66.3%)	39 (81.3%)	46 (69.7%)	0.21[a]
ER-	26.(36.1%)	29 (33.7%)	9 (18.8%)	20 (30.3%)	
Histologic grade					
Grade I	6 (8.3%)	10 (11.6%)	10 (20.8%)	6 (9.1%)	
Grade II	23 (31.9%)	29 (33.7%)	15 (31.3%)	30 (45.5%)	0.39[a]
Grade III	40 (55.6%)	42 (48.8%)	22 (45.8%)	27 (40.9%)	
Missing	3 (4.2%)	5 (5.8%)	1 (2.1%)	3 (4.6%)	
Tumor size					
0.1—≤2.0 cm	36 (50.0%)	47 (54.7%)	23 (47.9%)	26 (39.4%)	
>2.0—≤5.0 cm	24 (33.3%)	27 (31.4%)	20 (41.7%)	26 (39.4%)	0.28[a]
>5.0 cm	6 (8.3%)	7 (8.14%)	4 (8.3%)	12 (18.2%)	
Missing	6 (8.3%)	5 (5.8%)	1 (2.1%)	2 (3.0%)	
Stage					
I	21 (29.2%)	24 (27.9%)	16 (33.3%)	18 (27.3%)	
II	40 (55.6%)	41 (47.7%)	22 (45.8%)	32 (48.5%)	0.82[a]
III	11 (15.3%)	21 (24.42%)	10 (20.8%)	16 (24.2%)	
Year of Diagnosis					
1980-2004	27 (37.5%)	25 (29.1%)	15 (31.3%)	34 (51.5%)	0.03[a]
2005-present	45 (62.5%)	61 (70.9%)	33 (68.8%)	32 (48.5%)	
Mean BMI	24.6	26.3	26.1	27.3	0.01[b]
Chemotherapy					
Yes	48 (66.7%)	59 (68.6%)	34 (70.8%)	41 (62.1%)	
No	5 (6.9%)	7 (8.1%)	5 (10.4%)	6 (9.1%)	0.84[a]
Missing	19 (26.4%)	20 (23.3%)	9 (18.7%)	19 (28.8%)	
Radiation Therapy					
Yes	29 (40.3%)	38 (44.2%)	20 (41.7%)	29 (43.9%)	
No	20 (27.8%)	22 (25.6%)	11 (22.9%)	16 (24.2%)	0.99[a]
Missing	23 (32.0%)	26 (30.2%)	17 (35.4%)	21 (31.8%)	
Patients with Metastasis	7 (9.7%)	18 (20.9%)	6 (12.5%)	10 (15.2%)	0.24[b]

PPBC, Postpartum Breast Cancer; No., number of patients per group; ER, estrogen receptor; PR, progesterone receptor; DCIS, ductal carcinoma in situ; cm, centimeter; BMI, body mass index

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eTable 4 | University of Colorado metastasis-free survival analysis, stratified by biologic subtype					
UCH cohort using the old definition of luminal A/B (N=554)					
	Log Rank Test P value	**Univariate P value**	**Hazard Ratio [95% CI]**	**Multivariate P value***	**Hazard Ratio [95% CI]**
Group					
Luminal A		Reference	Reference	Reference	Reference
Luminal B	<0.001	0.900	1.04 [0.52-2.11]	0.94	1.03 [0.47-2.24]
Her2+		0.001	2.89 [1.54-5.44]	0.008	2.62 [1.28-5.35]
Triple Negative		0.002	2.39 [1.36-4.21]	0.004	2.44 [1.32-4.49]
Ki67 stained subset cohort using the old definition of luminal A/B (N=262)					
	Log Rank Test P value	**Univariate P value**	**Hazard Ratio [95% CI]**	**Multivariate P value***	**Hazard Ratio [95% CI]**
Group					
Luminal A		Reference	Reference	Reference	Reference
Luminal B	0.003	0.6700	0.79 [0.26-2.37]	0.72	0.82 [0.26-2.52]
Her2+		0.68	1.26 [0.42-3.81]	0.65	1.30 [0.42-4.03]
Triple Negative		0.002	3.29 [1.57-6.9]	0.004	3.27 [1.47-7.31]
Ki67 stained subset cohort using the updated definition of luminal A/B (N=258)					
	Log Rank Test P value	**Univariate P value**	**Hazard Ratio [95% CI]**	**Multivariate P value***	**Hazard Ratio [95% CI]**
Group					
Luminal A		Reference	Reference	Reference	Reference
Luminal B	0.007	0.44	1.51 [0.54-4.22]	0.35	1.71 [0.56-5.28]
Her2+		0.48	1.61 [0.43-6.05]	0.42	1.78 [0.44-7.29]
Triple Negative		0.007	4.21 [1.47-12.05]	0.01	4.42 [1.38-14.17]

***Multivariable Cox Proportional Hazards regression, adjusted for tumor size, age at diagnosis, and year of diagnosis**
95% CI = 95% confidence interval

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