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ER Assessment in Breast Cancer – An Update

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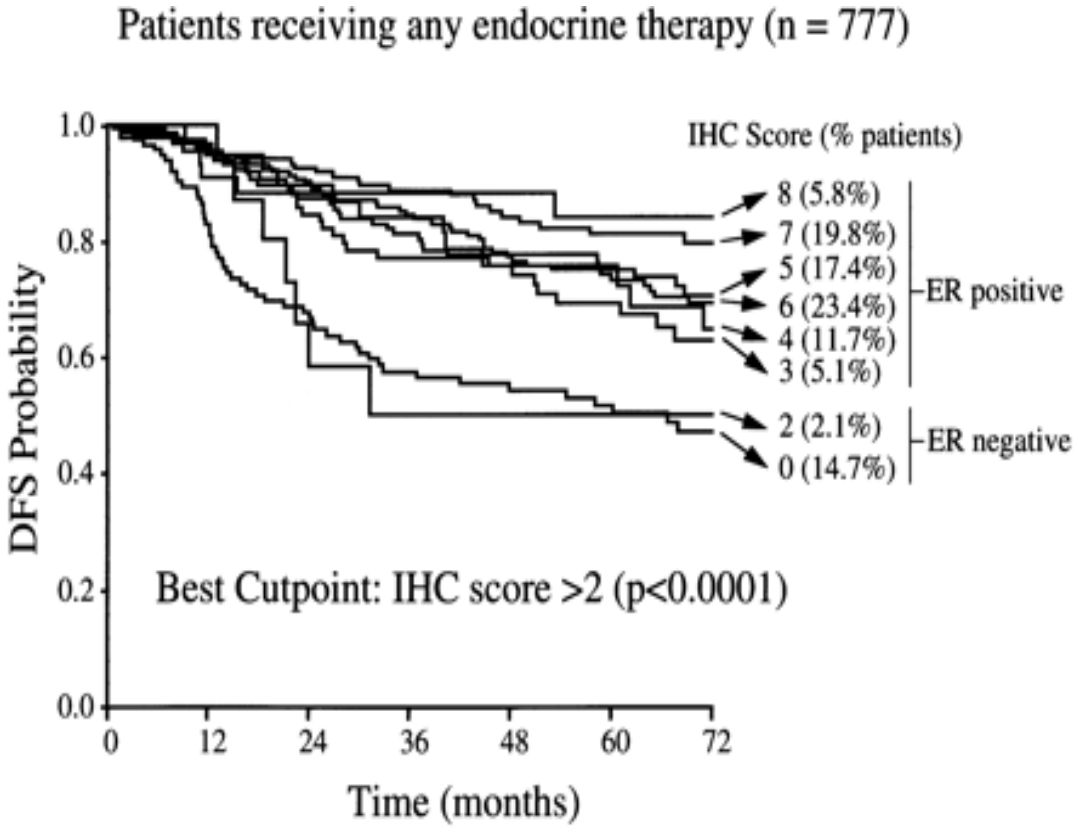
ER testing

ER low

ER low and therapy

Estrogen receptor α (ERα)

- A family of nuclear hormone receptor that act as ligand for activated transcription factors
- Binding of estrogen to ER leads to expression of genes involved in cell growth, differentiation and survival
- Expressed in 75-80% of breast cancers
- ER expression determined by IHC with 1% cutoff
- ER+ cancers derive substantial benefit from endocrine therapy but are associated with poorer chemotherapy response
- Higher level of ER expression predicts greater benefit from endocrine therapy, reduced cancer related mortality and recurrence



Harvey JM et al 1999 J Clin Oncol 17:1474

ER testing: Optimal algorithm

- ER/PR positive: 1-100% tumor nuclei positive
  - ER Low positive: 1-10% immunoreactivity
- ER/PR negative: <1% or 0% tumor nuclei positive
- Uninterpretable:
  - inadequate sample (insufficient tumor or severe artifacts present)
  - external and internal controls do not stain appropriately
  - pre-analytic variables have interfered
  - specimen has been decalcified using strong acids
- Specimen shows an ER-PR+ phenotype
  - need to rule out false ER- / PR+
- If sample is a cytology specimen, at least 100 cells should be counted

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Correlation of ER staining with histology

Table 3. Invasive Breast Cancer Histopathologic Concordance With ER Staining

Highly Unusual ER-Negative Results	Highly Unusual ER-Positive Results
Low-grade invasive carcinomas of no special type (also known as invasive ductal carcinoma)	Metaplastic carcinomas of all subtypes
Lobular carcinomas (classic type)	Adenoid cystic carcinomas and other salivary gland–like carcinomas of the breast
Pure tubular, cribriform, or mucinous carcinomas	Secretory carcinoma
Encapsulated papillary and solid papillary carcinomas	Carcinomas with apocrine differentiation

NOTE. If a result is considered highly unusual/discordant, additional steps should be taken to check the accuracy of the histologic type or grade as well as the preanalytic and analytic testing factors. This workup may include second reviews and repeat testing. If all results appear valid, the result can be reported with a comment noting that the findings are highly unusual and testing of additional samples may be of value to confirm the findings.

Abbreviation: ER, estrogen receptor.

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Recommendation for ER scoring

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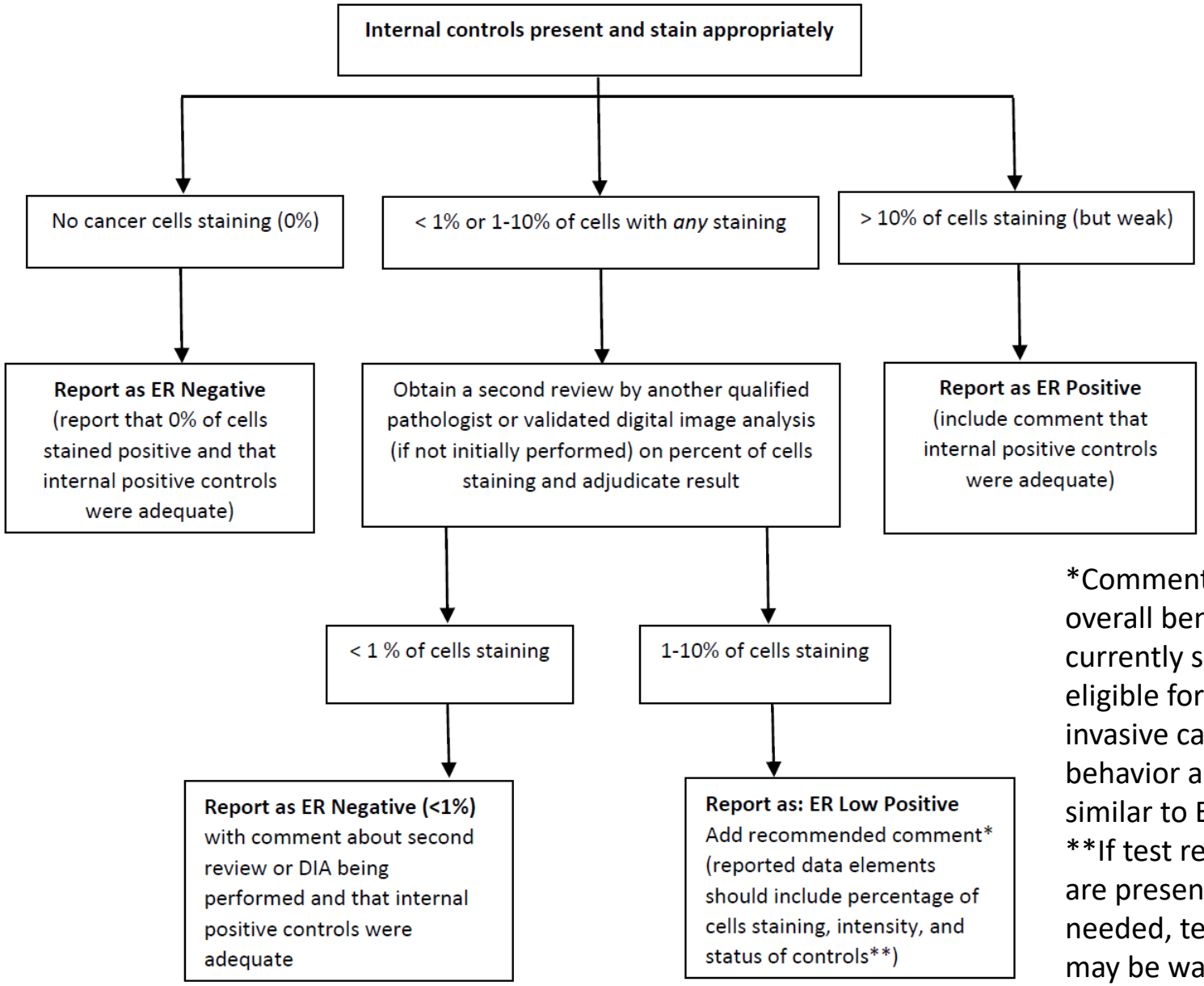

#Take steps to confirm/ adjudicate result per lab-specific SOP and correlate with histological result

*Comment on ER low expression: There are limited data on the overall benefit of endocrine therapies with these result, but they currently suggest possible benefit, so patients are considered eligible for endocrine treatment. There are data that suggest invasive cancers with these results were heterogeneous in both behavior and biology and often have gene expression profiles more similar to ER negative cancers.

**If test result are either ER negative or low and no internal controls are present, but external controls are appropriately positive. If needed, testing another specimen that contains internal controls may be warranted for confirmation of ER status.

Recommendation for ER scoring

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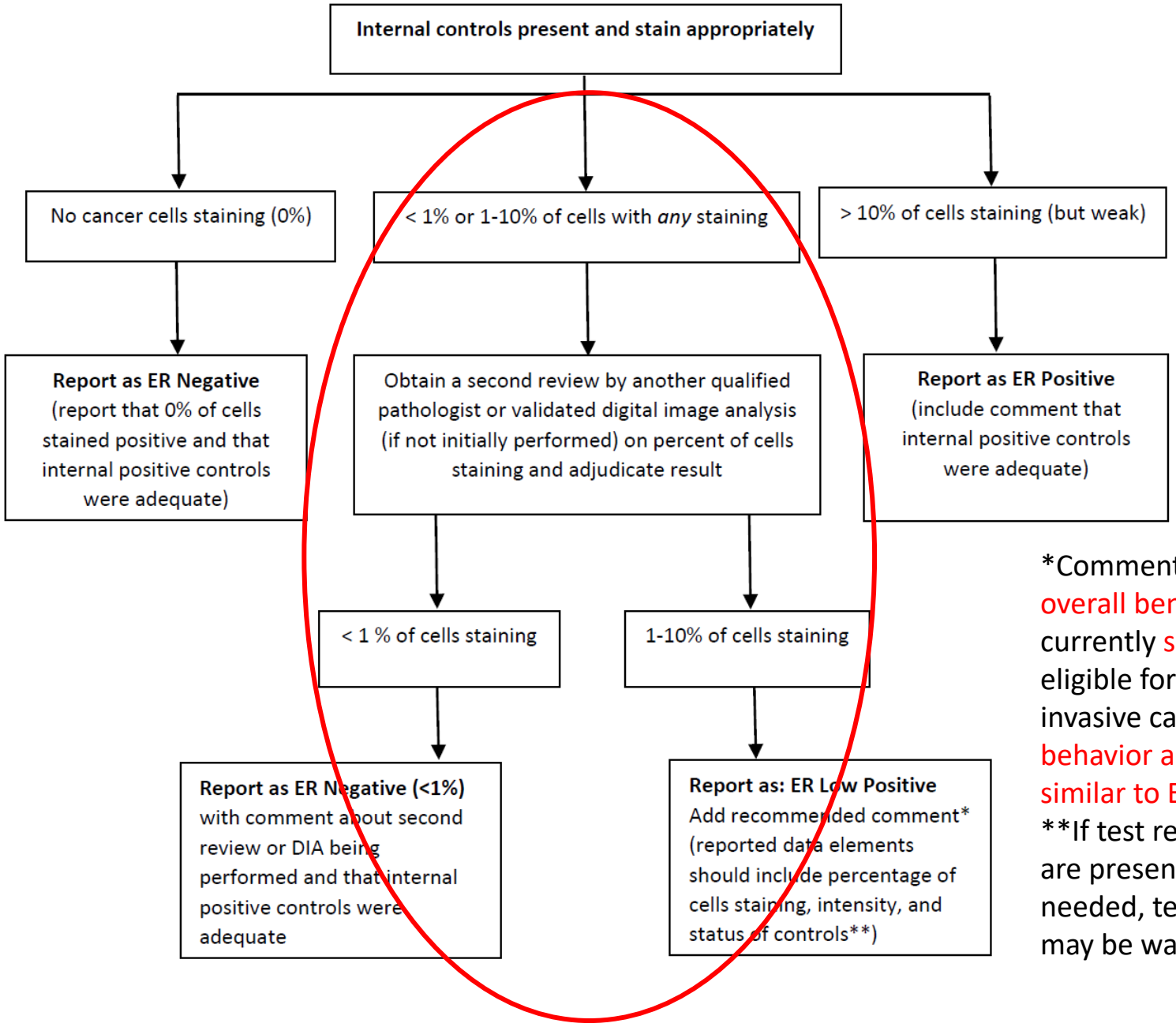

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**If test result are either ER negative or low and no internal controls are present, but external controls are appropriately positive. If needed, testing another specimen that contains internal controls may be warranted for confirmation of ER status.

ER testing

ER low

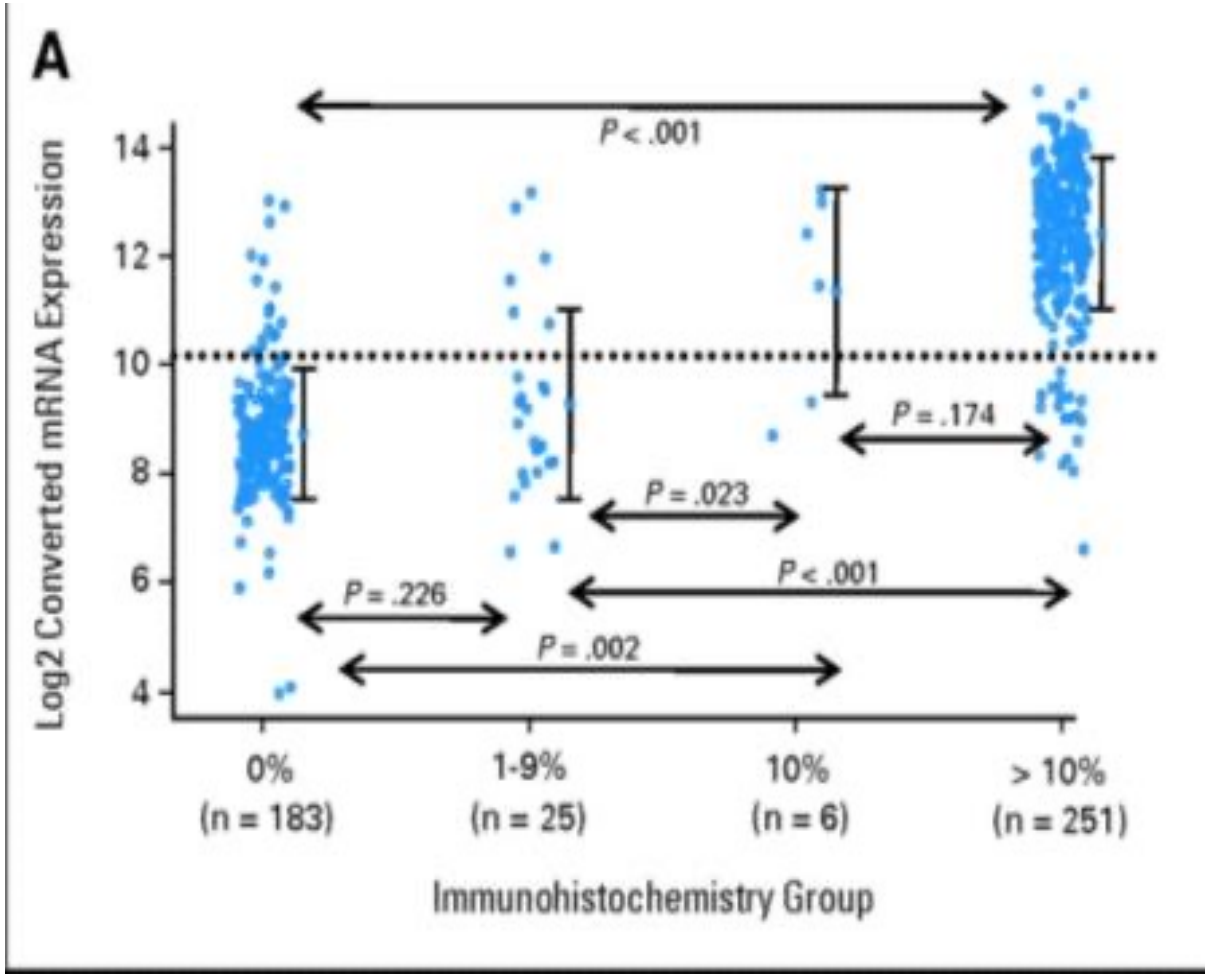
ER low and therapy

ER low

- Molecular evidence
- Molecular subtypes and ER low
- Oncotype Dx and RS
- Cut off 9% or 10%

ER low positive

- 1-9% weakly positive cells
- Accounts for 6% of all breast cancers
- Compared to ER high cancers, ER low cases were associated with younger age, higher stage, higher grade and HER2 positivity
- Showed more similar clinico-pathologic associations to ER negative cancers
- 24% ER low cases are *ESR1* mRNA positive (vs 67% and 92% for 10% and >10% ER positive), potentially endocrine sensitive
- Incidence of germline BRCA mutations in breast cancers with 1%-9% ER and/or PR was comparable to HR(-) group (36.1% vs 39.5%)



Iwamoto T et al 2012 J Clin Oncol 30:729
Deyarmin B et al 2013 Ann Surg Oncol 20:87
Sanford RA et al 2015 Cancer 121:3422

PAM50 classification (ER low-positive)

IHC (6F11) om CNB		Molecular Subtypes by PAM50				
IHC Level (%)	No. of Patients	Luminal A	Luminal B	HER2 Amplified	Basal	Normal
0	183	2 (1%)	1 (1%)	51 (28%)	111 (60%)	18 (10%)
1-9	25	0	2 (8%)	**8 (32%)**	**12 (48%)**	**3 (12%)**
10	6	2 (33%)	1 (16%)	1 (16%)	1 (16%)	1 (16%)
> 10	251	120 (48%)	61 (24%)	38 (15%)	16 (6%)	16 (6%)

Iwamoto T et al 2012 J Clin Oncol 30:729

Another study evaluated the molecular subtypes of HER2(-) ER-low breast cancers from two neoadjuvant trials (GeparQuinto and GeparSepto) by RNAseq showed

- 86.8% basal
- 10.5% HER2
- 2.6% normal like

Villegas SL et al 2021 Eur J Cancer 148:159

Table 1. Correlation of ER expression with clinicopathologic features and biomarkers.

	ER				*p*-value		
	Negative	Low	High	Total	All	Neg vs lo	Lo vs hi
Clinicopathologic features							
Age					0.014	0.005	0.004
Median	52	47	52	51			
IQR	45–61	44–54	45–62	45–61			
Range	23–101	22–82	27–97				
Tumour size					<0.001	0.782	0.003
Median	2.5	2.5	2.1	2.3			
IQR	2.0–3.5	2.1–6.7	1.5–3.0	1.6–3.3			
Range	0.1–13.0	1.1–8.0	0.1–10.2				
Grade					<0.001	0.014	<0.001
1	13 (2.6%)	3 (5.6%)	228 (18.0%)	244 (13.4%)			
2	99 (19.7%)	17 (31.5%)	641 (50.6%)	757 (41.5%)			
3	391 (77.7%)	34 (63.0%)	398 (31.4%)	823 (45.1%)			
Total	503	54	1267	1824			
Necrosis					<0.001	0.282	<0.001
Neg	270 (54.9%)	32 (62.7%)	1082 (87.8%)	1384 (77.9%)			
Pos	222 (45.1%)	19 (37.3%)	151 (12.2%)	392 (22.1%)			
Total	492	51	1233	1776			
LVI					0.040	0.024	0.011
Neg	355 (74.3%)	31 (59.6%)	913 (75.3%)	1299 (74.5%)			
Pos	123 (25.7%)	21 (40.4%)	300 (24.7%)	444 (25.5%)			
Total	478	52	1213	1743			
sTIL					<0.001	0.738	0.001
Low (>20%)	278 (68.0%)	32 (71.1%)	958 (89.3%)	1268 (83.0%)			
High (≤20%)	131 (32.0%)	13 (28.9%)	115 (10.7%)	259 (17.0%)			
Total	409	45	1073	1527			

	ER				*p*-value		
	Negative	Low	High	Total	All	Neg vs lo	Lo vs hi
CK5/6[c]					<0.001	0.005	0.039
Neg	343 (68.9%)	47 (87.0%)	1176 (94.0%)	1566 (86.9%)			
Pos	155 (31.1%)	7 (13.0%)	75 (6.0%)	237 (13.1%)			
Total	498	54	1251	1803			
CK14[c]					<0.001	0.005	1.00
Neg	424 (85.0%)	52 (98.1%)	1220 (97.4%)	1696 (94.0%)			
Pos	75 (15.0%)	1 (1.9%)	33 (2.6%)	109 (6.0%)			
Total	499	53	1253	1805			
AR[d]					<0.001	0.146	<0.001
Neg	240 (75.2%)	31 (86.1%)	323 (41.7%)	594 (52.6%)			
Pos	79 (24.8%)	5 (13.9%)	451 (58.3%)	535 (47.4%)			
Total	319	36	774	1129			
Ki67					<0.001	0.329	0.004
Low (<20%)	224 (44.9%)	28 (51.9%)	875 (69.6%)	1127 (62.6%)			
High (≥20%)	275 (55.1%)	26 (48.1%)	383 (30.4%)	674 (37.4%)			
Total	499	54	1258	1801			
HER2[b]					<0.001	0.358	<0.001
Neg	321 (64.1%)	38 (70.4%)	1134 (90.1%)	1493 (82.3%)			
Pos	180 (35.9%)	16 (29.6%)	125 (9.9%)	321 (17.7%)			
Total	501	54	1259	1814			
EGFR[c]					<0.001	0.118	<0.001
Neg	442 (88.8%)	44 (81.5%)	1238 (98.8%)	1724 (95.5%)			
Pos	56 (11.2%)	10 (18.5%)	15 (1.2%)	81 (4.5%)			
Total	498	54	1253	1805			
PR					<0.001	<0.001	<0.001
Neg	409 (82.1%)	13 (25.0%)	156 (12.4%)	578 (32.0%)			
1–20%	54 (10.9%)	15 (28.8%)	175 (13.9%)	244 (13.5%)			
>20%	35 (7.0%)	24 (46.2%)	928 (73.7%)	987 (54.6%)			
Total	498	52	1259	1809			
C-kit[c]					<0.001	0.133	0.227
Neg	384 (77.1%)	44 (86.3%)	1141 (91.2%)	1569 (87.2%)			
Pos	114 (22.9%)	7 (13.7%)	110 (8.8%)	231 (12.8%)			
Total	498	51	1251	1800			
P63[c]					<0.001	0.787	0.229
Neg	459 (92.2%)	50 (94.3%)	1216 (97.0%)	1725 (95.6%)			
Pos	39 (7.8%)	3 (5.7%)	38 (3.0%)	80 (4.4%)			
Total	498	53	1254	1805			

- ER low category (1-10%) showed a more similar clinico-pathologic and biomarker profile to ER negative than ER high cases
- younger age, larger tumor, higher proliferation, HER2 and basal marker expression

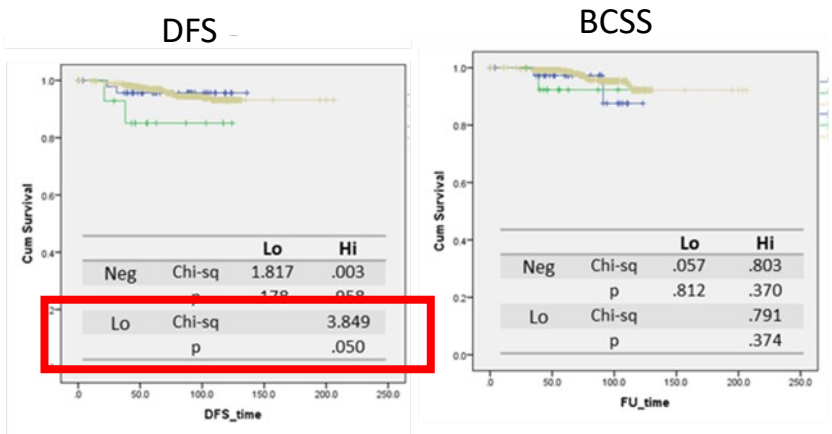
Poon IK et al 2020 Br J Cancer 123:1223

ER low cases in AJCC 8th staging

- ER low expression were staged as ER pos
- Many ER low cases were down-staged in pathological prognostic staging (compared to anatomical staging) in AJCC8
- Down-staging as per AJCC guideline for ER low category may incur a real possibility of risk underestimation and under treatment

	ER				p-value		
	Negative	low	High	Total	All	Neg vs lo	Lo Vs hi
AS					<.001	.258	.007
IA	97 (20.5%)	8 (16.0%)	346 (29.3%)	451 (26.5%)			
IB	2 (0.4%)	1 (2.0%)	15 (1.3%)	18 (1.1%)			
IIA	172 (36.4%)	10 (20.0%)	385 (32.7%)	567 (33.3%)			
IIB	79 (16.7%)	15 (30.0%)	188 (15.9%)	282 (16.6%)			
IIIA	62 (13.1%)	11 (22.0%)	153 (13.0%)	226 (13.3%)			
IIIB	13 (2.7%)	0 (0%)	11 (0.9%)	24 (1.4%)			
IIIC	48 (10.1%)	5 (10.0%)	81 (6.9%	134 (7.9%)			
Total	473	50	1179	1702			
PPS					<.001	<.001	.003
IA	51 (10.8%)	15 (30.6%)	635 (54.0%)	701 (41.3%)			
IB	54 (11.4%)	12 (24.5%)	241 (20.5%)	307 (18.1%)			
IIA	167 (35.4%)	7 (14.3%)	118 (10.0%)	292 (17.2%)			
IIB	40 (8.5%)	9 (18.4%)	69 (5.9%)	118 (7.0%)			
IIIA	74 (15.7%)	2 (4.1%)	57 (4.9%)	133 (7.8%)			
IIIB	31 (6.6%)	4 (8.2%)	44 (3.7%)	79 (4.7%)			
IIIC	55 (11.7%)	0 (0%)	11 (0.9%)	66 (3.9%)			

PPS IA

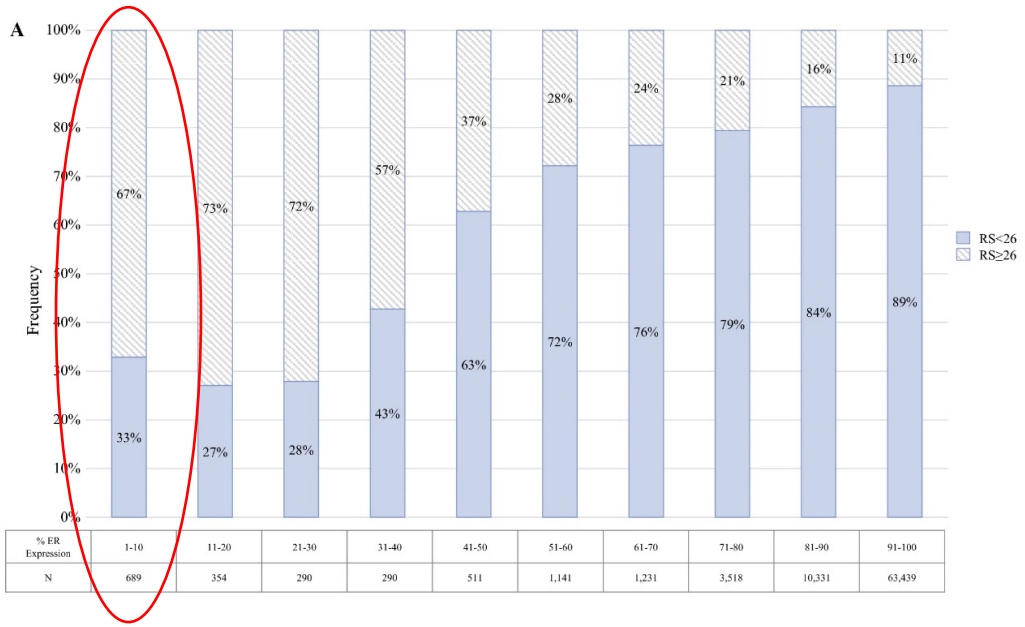


Poon IK et al 2020 Br J Cancer 123:1223

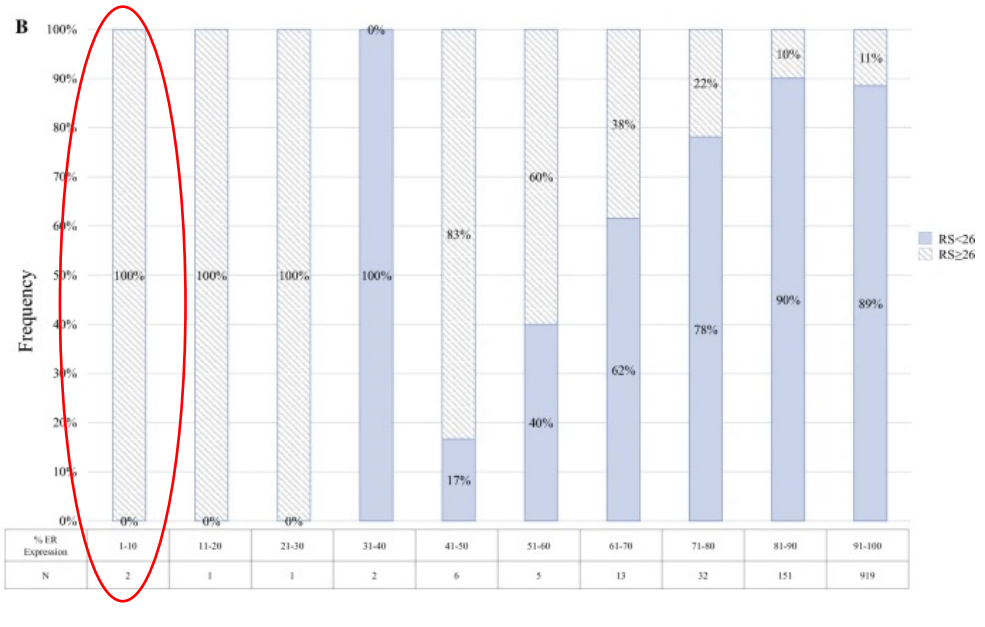
Recurrence Score according to ER expression

- Approximately one-third and none of the ER 1–10% in NCDB and DFBCCDB cohort, respectively, had an RS <26
- ER1-10% tumors would benefit from chemotherapy

National Cancer Database cohort (2018–2019)



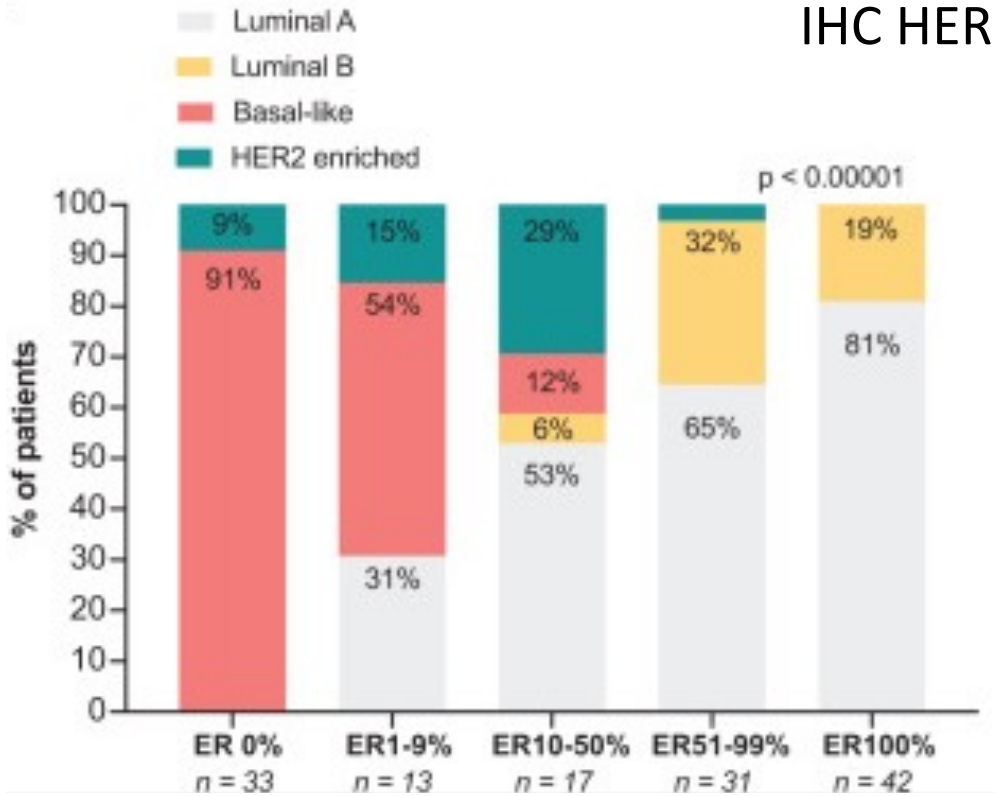
Dana-Farber Brigham Cancer Center Database (2016–2021)

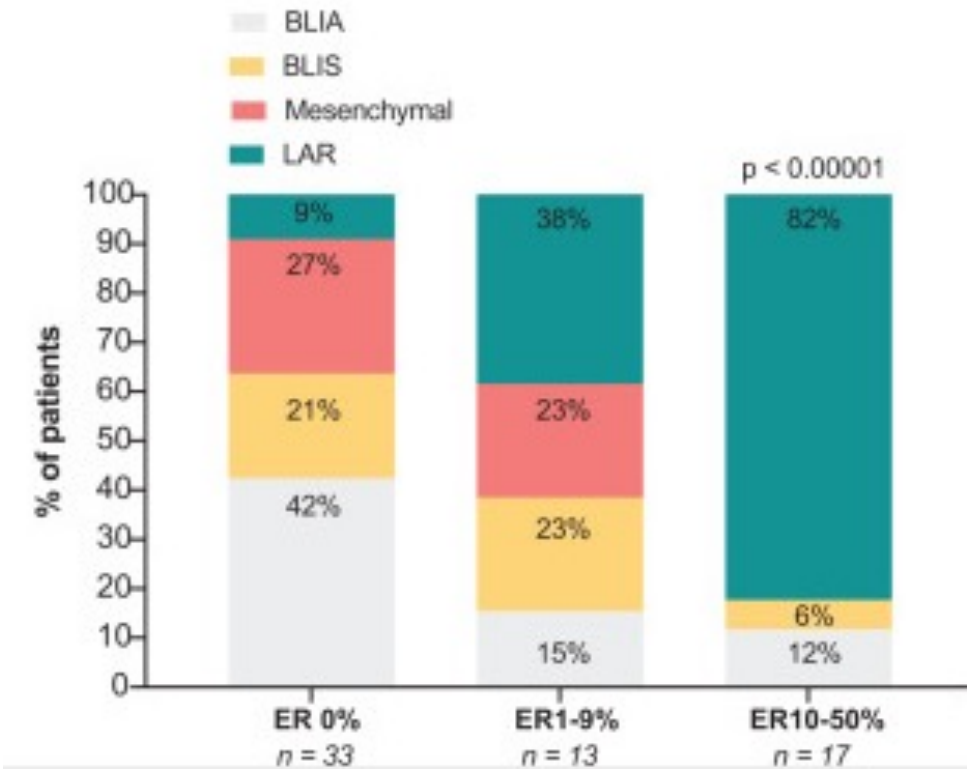


Higgins T et al 2024 Ann Surg Oncol 31:2244

Molecular subtypes according to ER expression level

IHC HER2 neg





- Proportion of basal-like tumors decreased with increased ER level :
- 0% (91%), 1–9% (54%), 10–50% (12%), 51-99% (3%), 100% (0%)

- Tumors with a BLIA TNBC subtype were restricted to ER expression of 50% or lower

Voorwerk L et al 2023 NPJ Breast Cancer

Variables in model	Patients (N)	Events (N)	Model 1		Model 2	
			HR (95% CI)	95% CI	HR (95% CI)	95% CI
ER status						
ER-low (ER 1–9%)	468	101	**ref.**		**ref.**	
ER-negative (ER 0%)	4260	1018	1.13	0.91–1.39	1.11	0.90–1.36
Histological subtype						
Ductal	3928	951	**ref.**		**ref.**	
Lobular	100	35	1.10	0.77–1.58	1.04	0.72–1.48
Mixed	700	133	0.83	0.69–1.00	0.83	0.69–1.00
Grade (NHG)						
I	99	16	**ref.**		**ref.**	
II	938	197	1.23	0.74–2.06	1.36	0.81–2.28
III	3691	906	1.47	0.89–2.42	1.76	1.07–2.90
TNM stage (clin/path)[a]						
I	1950	258	**ref.**		**ref.**	
II	2252	557	1.85	1.59–2.15	1.96	1.68–2.28
III	526	304	5.25	4.41–6.25	5.79	4.86–6.90
Chemotherapy						
No	1547	636	Not incl		**ref.**	
Yes	3181	483	Not incl		0.50	0.43–0.58
Age						
<40	378	55	**ref.**		**ref.**	
40–49	705	118	1.14	0.83–1.57	1.11	0.80–1.53
50–64	1487	214	1.04	0.78–1.41	1.01	0.75–1.36
65–79	1596	391	2.02	1.52–2.68	1.80	1.35–2.39
≧80	562	341	5.49	4.11–7.32	3.33	2.44–4.53
Year of diagnosis						
2008–2011	906	331	**ref.**		**ref.**	
2012–2016	1667	461	1.02	0.87–1.18	1.28	1.09–1.51
2017–2020	2155	327	1.06	0.90–1.26	1.38	1.15–1.66

Model 1: adjusted for ER-status, histological subtype, grade, stage, age and year of diagnosis. **Model 2**: adjusted for ER-status, histological subtype, grade, stage, age, year of diagnosis and chemotherapy. [a]cT and cN/pN were used for patients with neoadjuvant treatment.

Table 3: **Multivariable Cox regression analysis of prognostic factors associated with overall survival in a poulation-based cohort of 4728 women with ER-negative or ER-low primary breast cancer diagnosed between 2008 and 2020.**

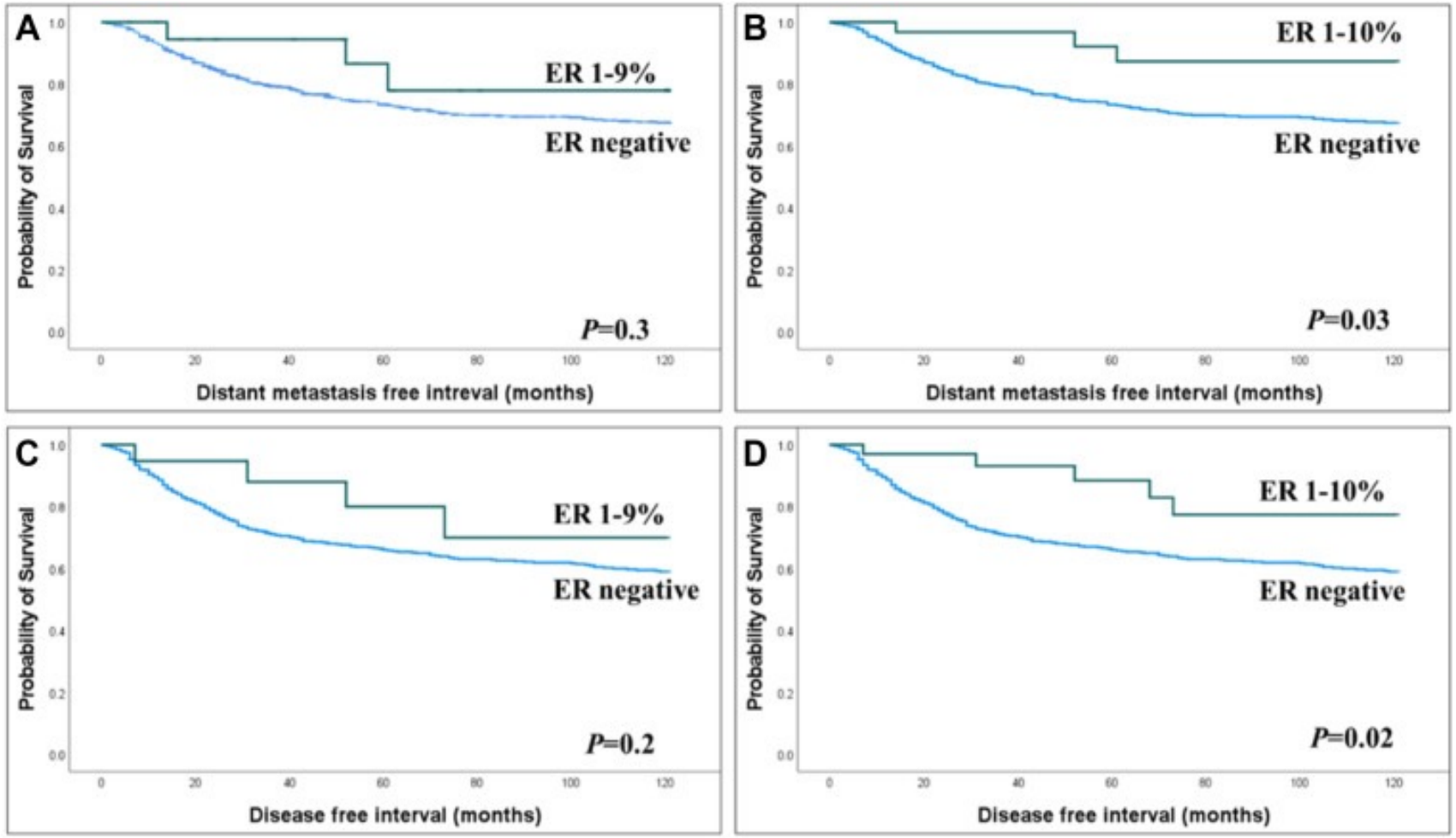
IHC HER2 neg
TNBC by IHC (10% cut off)

- No association between ER-status and OS in the multivariable analysis
- Model 1 : adjustment for stage, age, grade and year of diagnosis
- Model 2 : adjustment also for chemotherapy

ER low outcome similar to ER neg

Acs B et al 2024 Lancet Reg Health -Eur

ER 10% as ER-low or ER-positive?



Patients with ER 1-10% had both longer DMFS and DFS compared with ER neg patients

ER 1-9% : no difference with ER neg

Makhlouf S et al 2023 Mod Pathol

ER 10% as ER-low or ER-positive?

- ER 10% cancers had significantly lower grade, better NPI, and more PR positivity than tumors with ER 1-9%, but did not show any significant difference from tumors with ER 11-30%
- ER-low 1-9% were similar to the ER neg tumors but showed significant differences compared with ER pos tumors (≥10%) in most clinicopathologic parameters

Makhlouf S et al 2023 Mod Pathol

Characteristics	ER IHC expression in needle core biopsies				
	ER 10%	ER 1-9%	X^2 (*p*-value) [a]	ER 11-30%	X^2 (*p*-value) [b]
Age at diagnosis (years)					
< 50	13 (28%)	41 (33%)	0.5 (0.4)	52 (43%)	3.5 (0.06)
≥ 50	34 (72%)	82 (67%)		68 (57%)	
Tumour size (cm)					
< 2	34 (72%)	58 (47%)	8.7 (**0.003**)	70 (58%)	2.8 (0.09)
≥ 2	13 (28%)	65 (53%)		50 (42%)	
Tumour grade					
1	4 (8%)	4 (3%)	13.8 (**0.001**)	13 (11%)	3.9 (0.1)
2	21 (46%)	23 (20%)		35 (29%)	
3	21 (46%)	88 (77%)		72 (60%)	
Mitotic count					
1	18 (39%)	19 (16%)	12 (**0.002**)	38 (32%)	4.8 (0.08)
2	15 (33%)	34 (30%)		26 (22%)	
3	13 (28%)	62 (54%)		56 (46%)	
Nuclear pleomorphism					
1	0	2 (2%)	4 (0.1)	0	0.4 (0.5)
2	9 (20%)	11 (9%)		29 (24%)	
3	37 (80%)	102 (89%)		91 (76%)	
Tubule formation					
1	4 (9%)	2 (2%)	4.9 (0.08)	9 (7%)	0.11 (0.9)
2	11 (24%)	21 (18%)		31(26%)	
3	31 (61%)	92 (80%)		80 (67%)	
Nottingham Prognostic Index					
Good Prognostic Group	11 (25%)	10 (9%)	7 (**0.03**)	32 (27%)	0.9 (0.6)
Moderate Prognostic Group	26 (59%)	82 (73%)		62 (52%)	
Poor Prognostic Group	7 (16%)	20 (18%)		26 (21%)	
Histological types					
No special type (NST)	33 (72%)	100 (87%)	5 (0.1)	90 (75%)	0.2 (0.9)
Lobular	4 (9%)	4 (3%)		8 (7%)	
Other special types	3 (6%)	3 (3%)		7 (6%)	
Mixed NST and other tumour types	6 (13%)	8 (7%)		15 (12%)	
Axillary nodal status					
Negative	25 (56%)	74 (66%)	1.3 (0.2)	74 (62%)	0.5 (0.4)
Positive	20 (44%)	39 (34%)		46 (38%)	
Lymph node stage					
1 (Negative)	25 (56%)	74 (65%)	1.4 (0.4)	74 (62%)	1.5 (0.4)
2 (1-3 positive)	15 (33%)	28 (25%)		29 (24%)	
3 (>3 positive)	5 (11%)	11 (10%)		17 (14%)	
Lymphovascular Invasion					
Negative	36 (80%)	94 (83%)	0.22 (0.6)	87 (73%)	0.97 (0.3)
Positive	9 (20%)	19 (17%)		33 (27%)	
Progesterone receptor					
Negative	26 (58%)	82 (82%)	9.5 (**0.002**)	55 (47%)	1.4 (0.2)
Positive	19 (42%)	18 (18%)		61 (53%)	
HER2					
Negative	27 (60%)	72 (69%)	1.2 (0.2)	81 (70%)	1.6 (0.2)
Positive	18 (40%)	33 (31%)		34 (30%)	
Ki67 index					
Low (≤14%)	6 (46%)	3 (14%)	4.1 (**0.04**)	19 (35%)	0.53 (0.3)
High (>14%)	7 (54%)	18 (86%)		35 (65%)	

[a] ER 10% vs ER 1-9%

[b] ER 10% vs ER 11-30%

PAM50 classification : ER 1-9% and ER 10%

IHC		Molecular Subtypes by PAM50				
IHC Level (%)	No. of Patients	Luminal A	Luminal B	HER2 Amplified	Basal	Normal
0	183	**2 (1%)**	1 (1%)	51 (28%)	**111 (60%)**	18 (10%)
1-9	25	**0**	2 (8%)	8 (32%)	**12 (48%)**	3 (12%)
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Iwamoto T et al 2012 J Clin Oncol 30:729

ER testing

ER low

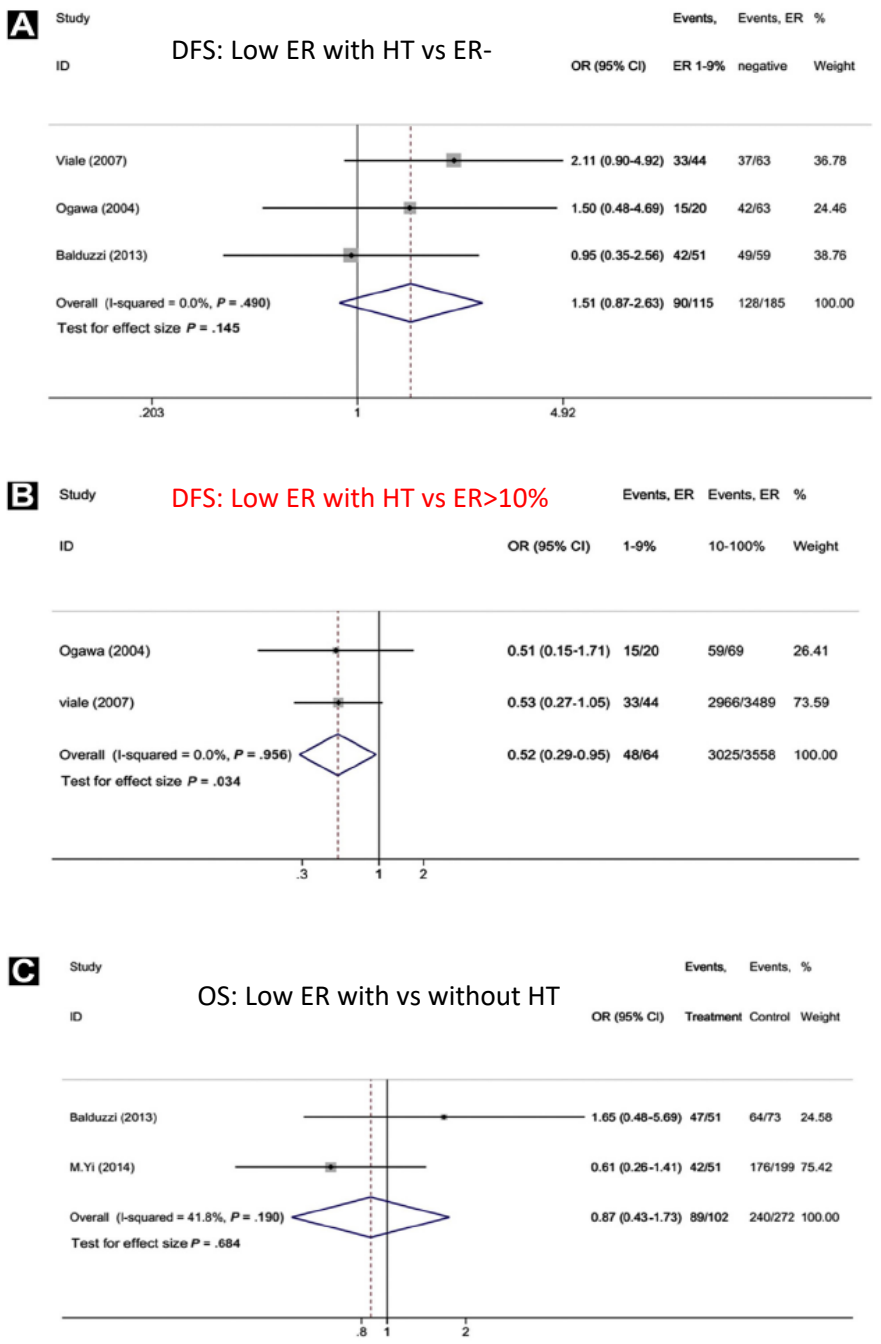
ER low and therapy

ER low and endocrine therapy

Six retrospective studies with 16,606 patients, including patients of ER low (1-9%), ER neg and ER high (≥10%) (N = 834, 4176, 11596 respectively)

- Patients with ER high - significantly better prognosis
- ER low with HT had a slightly better prognosis than ER neg group
  - 5 year DFS pooled OR 1.47
  - 5 year OS pooled OR 1.23
  - No significant differences in RFS
- No significant differences in 5 year OS for ER low patients with or without endocrine therapy

ER low biologically similar to ER neg

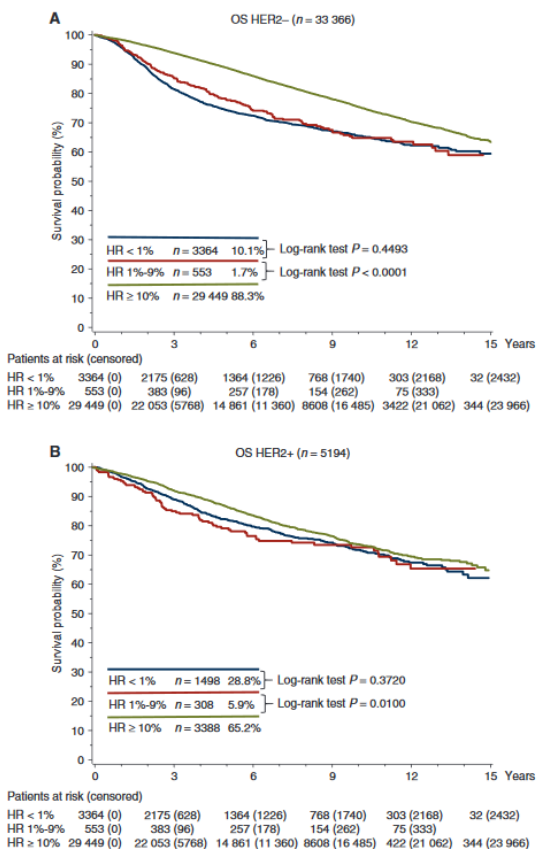


Chen T et al 2018 Clin Breast Cancer 18:1

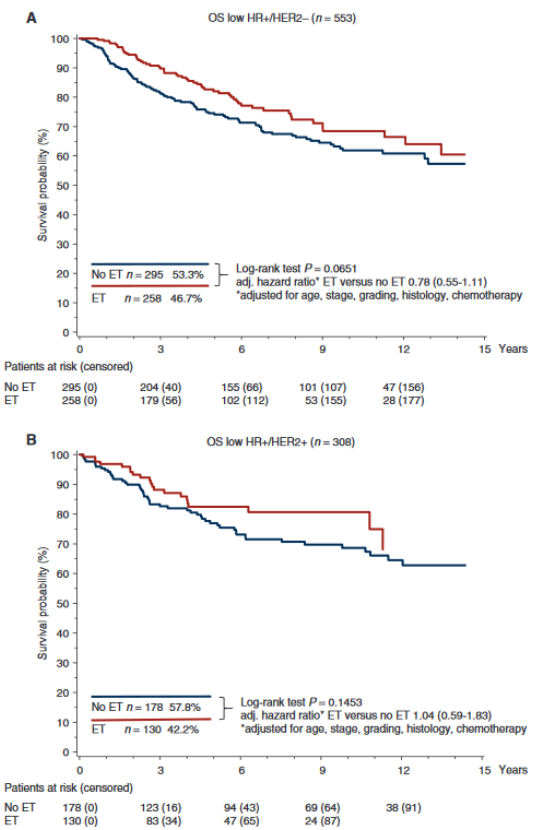
HR-low breast cancer: HER2(-) and HER2(+) cohorts

- 15 year cohort
- Tumor characteristics between the HR low (N=861) compared to HR high (N=32837) or HR neg (N=4862) groups were similar irrespective to HER2 status
- In the HER2 neg cohort prognosis of HR low positive tumors was similar to that of HR neg tumors and differed significantly with HR high tumors
  - OS hazard ratio for HR high : 0.66 (95% CI 0.55-0.78)
- In the HER2 pos cohort, the differences between HR low/neg and HR high were less pronounced
  - HR expression have a lower impact on outcome
  - may be due to anti-HER2 therapy
- Patients with HR low tumors seem to benefit only slightly from endocrine therapy, but this difference was not statistically different

different HR level



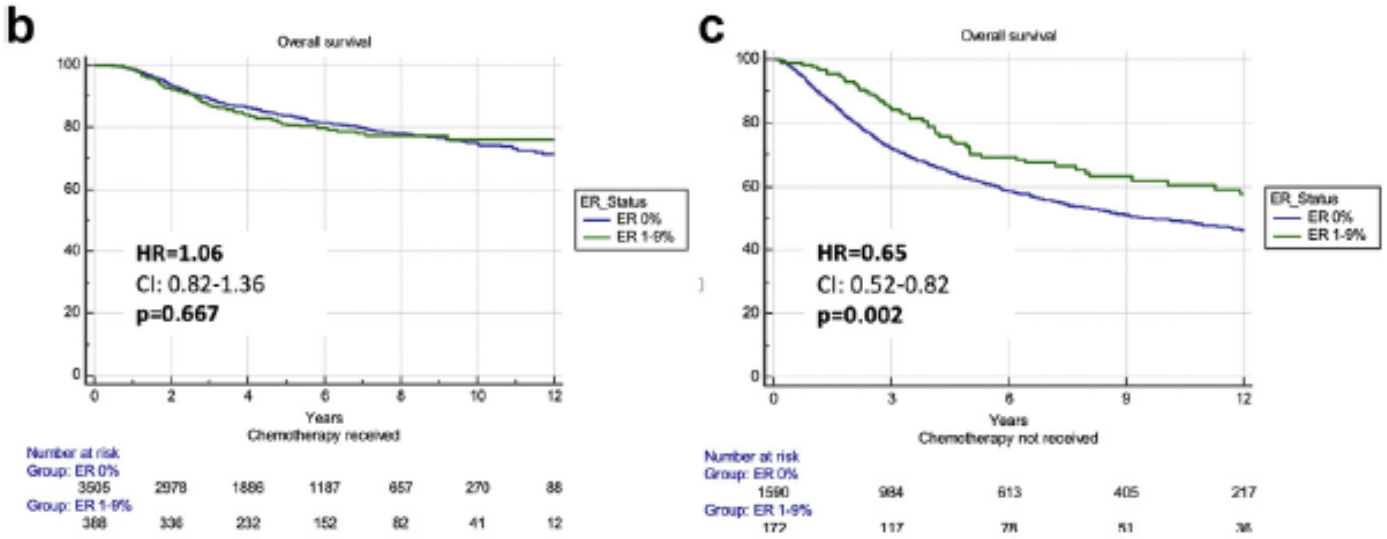
HR-low with/without endocrine therapy



Schrodi S et al 2021 Ann Oncol 32:1410

ER zero Vs ER low HER2 neg Breast Cancer (chemo)

- Swedish population-based cohort study
- 5655 ER(<10%) HER2-ve tumors, around 10% ER-low and 90% ER-zero
- ER-low showed fewer grade III tumor (69.4% vs 80.8%), lower median Ki67 (60% vs 63%), more HER2 2+ (21.9% vs 13.6%) and more lobular BC (6.8% vs 1.6%) than ER-zero HER2-ve BC
  - No differences in tumor size, nodal status and treatment received (except endocrine therapy)
- ER-low HER2-ve had similar prognosis for patients with chemotherapy
  - In women given chemotherapy, there was no difference in OS (HR 1.06, 95% CI 0.82–1.36)
  - In women not given chemotherapy, those with ER-low tumor had a statistically significantly better OS than those with ER-zero disease (HR 0.65, 95% CI 0.52–0.82)

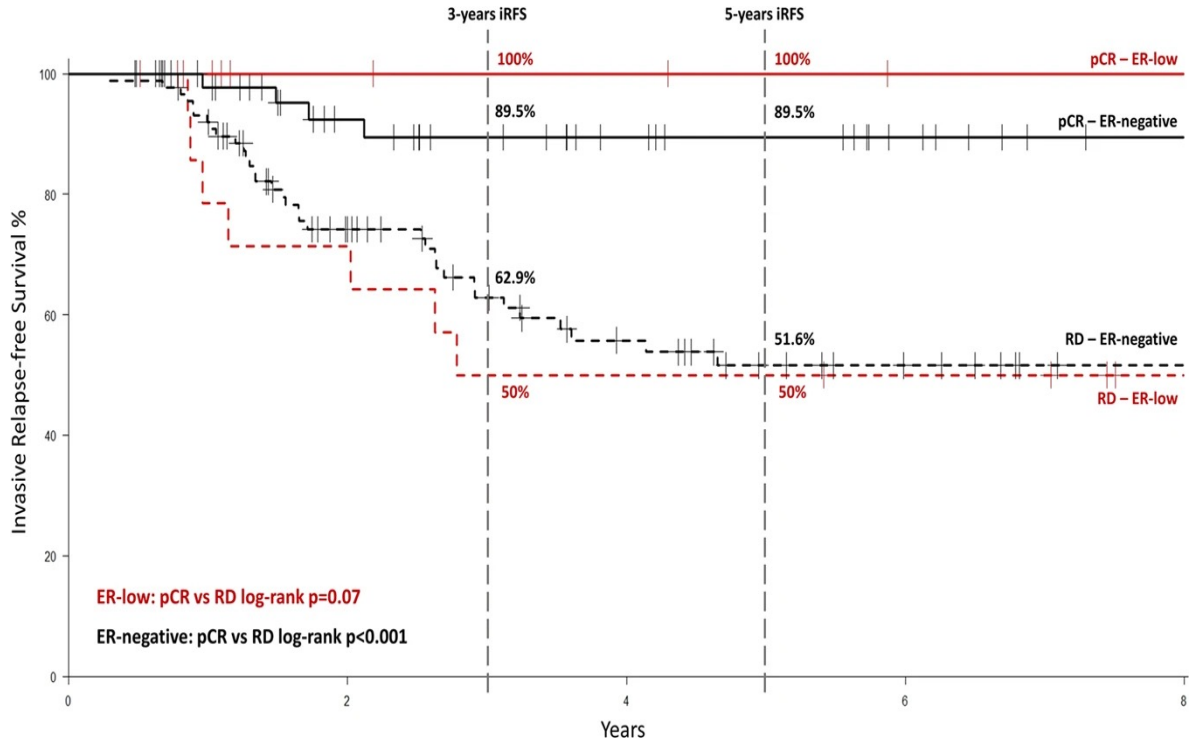


Acs B et al 2024 Lancet Reg Health -Eur

ER-low vs ER-zero in neoadjuvant chemotherapy

- Primary breast cancers with ER1–9% shows similar outcome to ER<1% with NAC
- pCR rate (ER-low vs –neg): 44% vs 38% (reported pCR rate for ER-pos: 7-16%)
- 5-yr OS : 82.3% vs 76.7%; 5-yr invasive RFS : 74.0% vs 73.1%
- Median FU of 54 months

	iRFS		DRFS		OS	
	Univariate	Multivariate	Univariate	Multivariate	Univariate	Multivariate
	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)
Age (continuous)	1.01 (0.99–1.03)	–	1.02 (1.00–1.04)	1.03 (1.01–1.04)	1.02 (1.00–1.04)	1.03 (1.01–1.04)
Grades						
G1–G2	Ref	–	Ref	–	Ref	–
G3	1.00 (0.54–1.86)	–	1.08 (0.55–2.11)	–	1.65 (0.70–3.85)	–
Ki67 baseline (continuous)	1.00 (0.99–1.01)	–	0.99 (0.98–1.00)	–	1.00 (0.99–1.01)	–
Stages						
I	Ref	Ref	Ref	Ref	Ref	Ref
II	1.93 (1.07–3.47)	1.93 (1.07–3.48)	1.45 (0.79–2.67)	1.67 (0.90–3.09)	1.33 (0.70–2.51)	1.53 (0.81–2.93)
III	4.59 (2.45–8.60)	4.60 (2.44–8.65)	4.01 (2.12–7.57)	4.73 (2.48–9.02)	3.02 (1.53–5.99)	3.59 (1.79–7.21)
ER expression (continuous)	1.00 (0.78–1.28)	–	1.04 (0.80–1.34)	–	1.02 (0.77–1.36)	–
ER expression						
<1%	Ref	Ref	Ref	Ref	Ref	Ref
1–9%	1.20 (0.62–2.32)	0.98 (0.51–1.91)	1.26 (0.63–2.52)	1.21 (0.59–2.47)	1.10 (0.50–2.40)	1.06 (0.48–2.36)



Dieci M et al 2021 npj breast cancer 7:101

TILs density in different ER status

Table 1. Correlation of ER expression with clinicopathologic features and biomarkers.

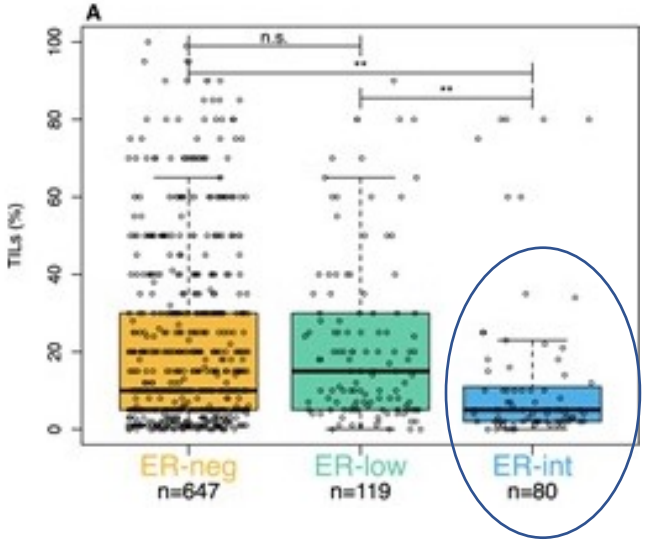
	ER				*p*-value		
	Negative	Low	High	Total	All	Neg vs lo	Lo vs hi
Clinicopathologic features							
Age					0.014	0.005	0.004
Median	52	47	52	51			
IQR	45–61	44–54	45–62	45–61			
Range	23–101	22–82	27–97				
Tumour size					<0.001	0.782	0.003
Median	2.5	2.5	2.1	2.3			
IQR	2.0–3.5	2.1–6.7	1.5–3.0	1.6–3.3			
Range	0.1–13.0	1.1–8.0	0.1–10.2				
Grade					<0.001	0.014	<0.001
1	13 (2.6%)	3 (5.6%)	228 (18.0%)	244 (13.4%)			
2	99 (19.7%)	17 (31.5%)	641 (50.6%)	757 (41.5%)			
3	391 (77.7%)	34 (63.0%)	398 (31.4%)	823 (45.1%)			
Total	503	54	1267	1824			
Necrosis					<0.001	0.282	<0.001
Neg	270 (54.9%)	32 (62.7%)	1082 (87.8%)	1384 (77.9%)			
Pos	222 (45.1%)	19 (37.3%)	151 (12.2%)	392 (22.1%)			
Total	492	51	1233	1776			
LVI					0.040	0.024	0.011
Neg	355 (74.3%)	31 (59.6%)	913 (75.3%)	1299 (74.5%)			
Pos	123 (25.7%)	21 (40.4%)	300 (24.7%)	444 (25.5%)			
Total	478	52	1213	1743			
sTIL					<0.001	0.738	0.001
Low (>20%)	278 (68.0%)	32 (71.1%)	958 (89.3%)	1268 (83.0%)			
High (≤20%)	131 (32.0%)	13 (28.9%)	115 (10.7%)	259 (17.0%)			
Total	409	45	1073	1527			

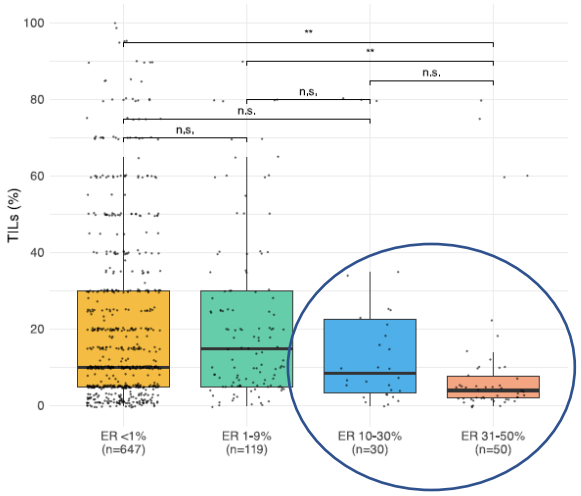
- ER low category showed higher TIL than ER high BC

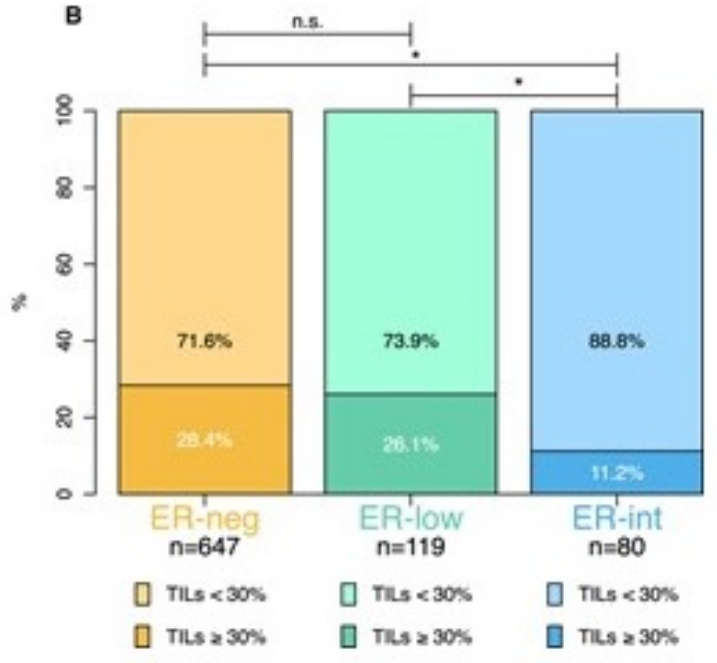
Poon IK et al 2020 Br J Cancer 123:1223

TILs density in different ER status

- TILs were similar in ER-neg and ER-low (1-9%) (median[IQR] 10% [5-30] vs 15% [5-30]); but significantly higher than ER-int (10-50%)
- The differences with ER-int mainly found in those with ER 31-50
- EER-int subgroup (10-30%) showed no significant difference compared with ER-neg and ER-low tumors
- Similar proportions of patients with high TILs (≥30%) were observed in ER-neg and ER-low groups (28.4% vs 26.1%), but a lower proportion in ER-int patients (11.2%)



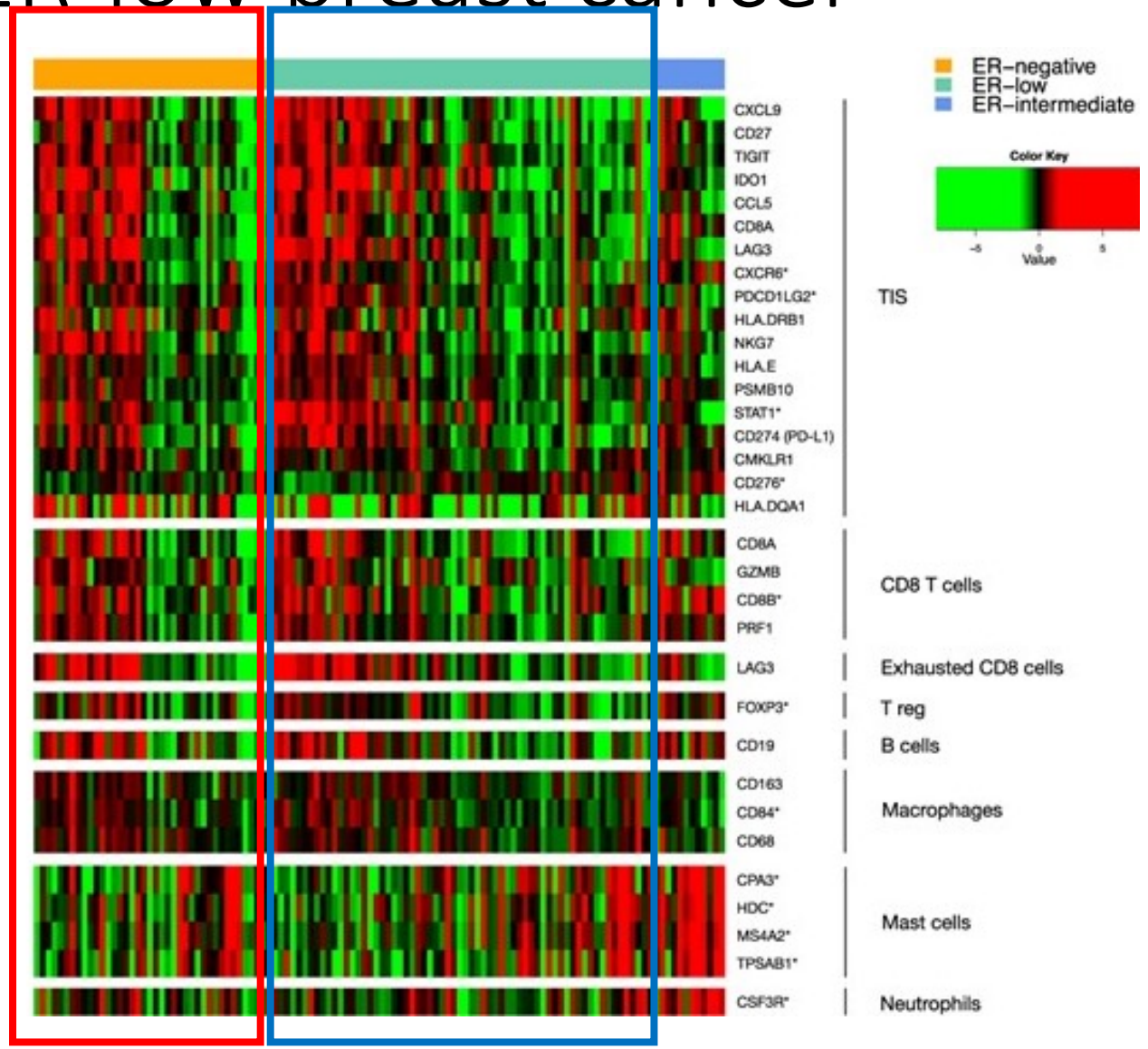




Massa D et al 2024 JNCI

Immune landscape of ER-low breast cancer

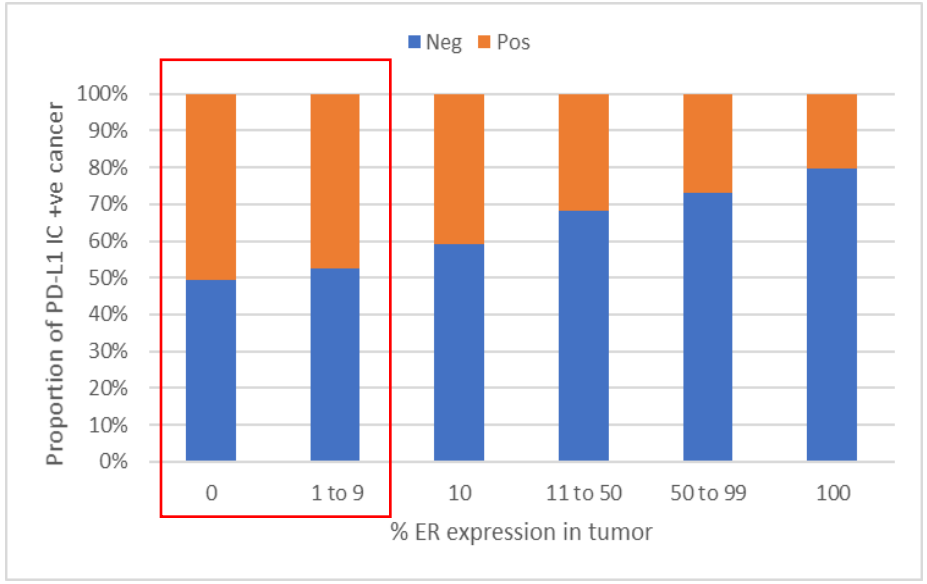
- ER-low and ER-neg tumors showed little differences
  - Only 3 of 766 genes were differentially expressed in ER low compared with ER neg tumors, showing upregulation of *GATA3* and downregulation of *EDN1* and *PROM1*
  - No significant differences in 164 immune-related gene (antigen presentation, cytokine / chemokine signaling, immune infiltration, TGF-beta signaling or the characterization of immune cells)
- ER low has a distinct expression pattern compared with ER int tumors
- ER int tumors displayed a distinct immune profile - increased expression of some mast cell-related genes.
  - MCs showed to hinder activation of cMET and promote expression and activation of ER and other luminal markers



Massa D et al 2024 JNCI; Majorini MT et al 2020 Cancer Res

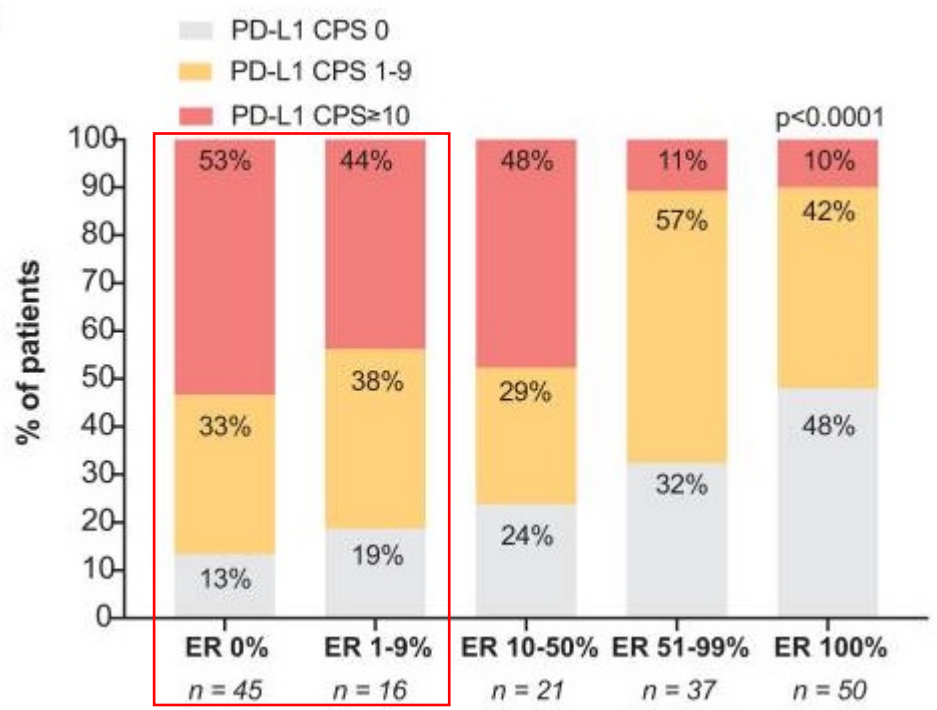
PD-L1 expression according to ER level

ER-low (1–9%) are comparable to ER-negative tumors in terms of PD-L1 expression



Tse G et al unpublished data

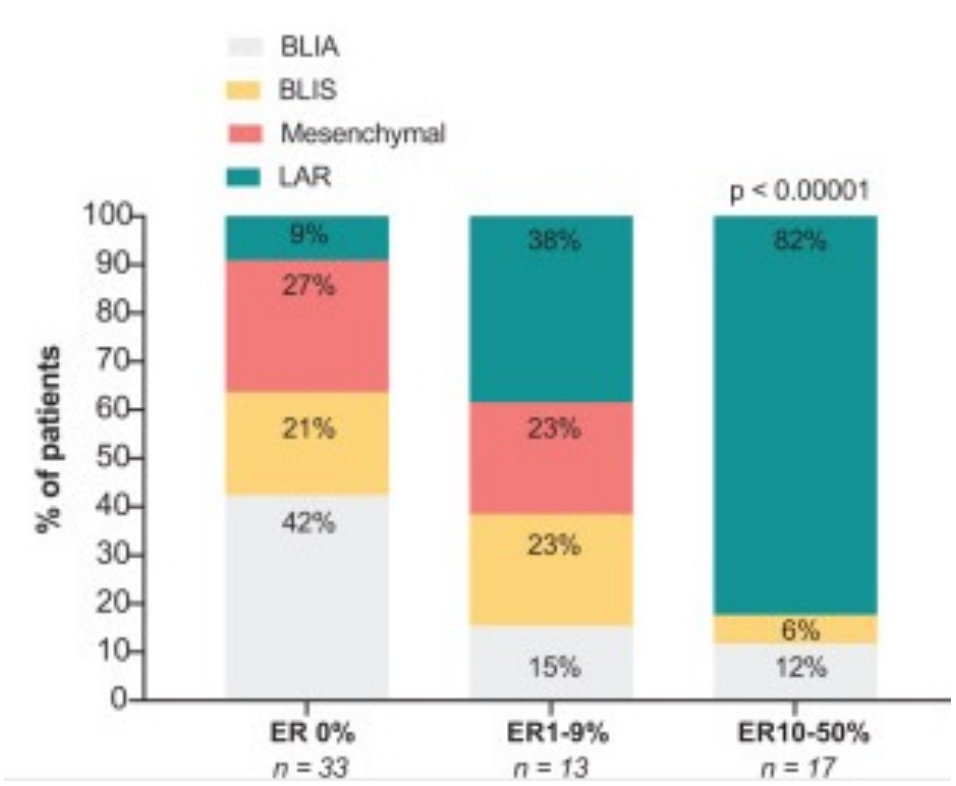
- PD-L1 clone SP142
- Positivity defined by any PD-L1 expression in immune cells (IC)

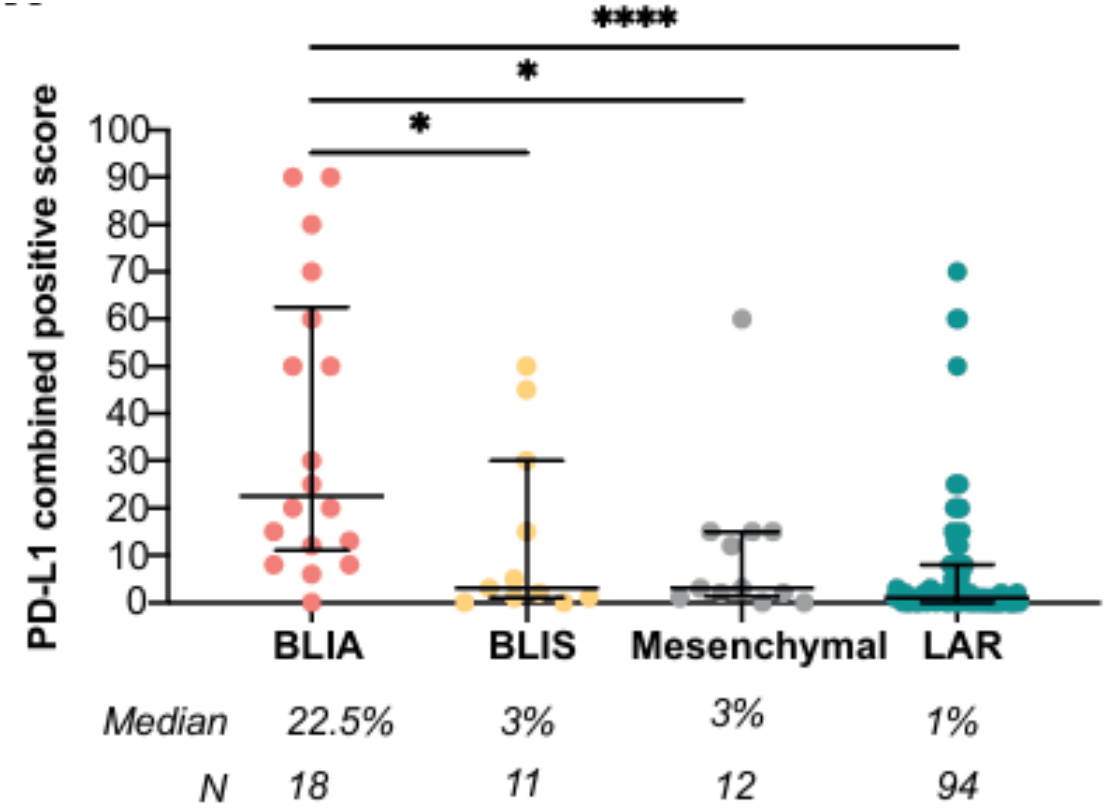


Voorwerk L et al 2023 NPJ Breast Cancer

- PD-L1 clone 22C3
- CPS = [the number of PD-L1+ve cells (tumor cells and immune cells)/ total number of tumor cells] *100

PD-L1 expression in TNBC profiling subtypes





PD-L1 expression was highest in the BLIA tumors and associated with response to ICB

Voorwerk L et al 2023 NPJ Breast Cancer

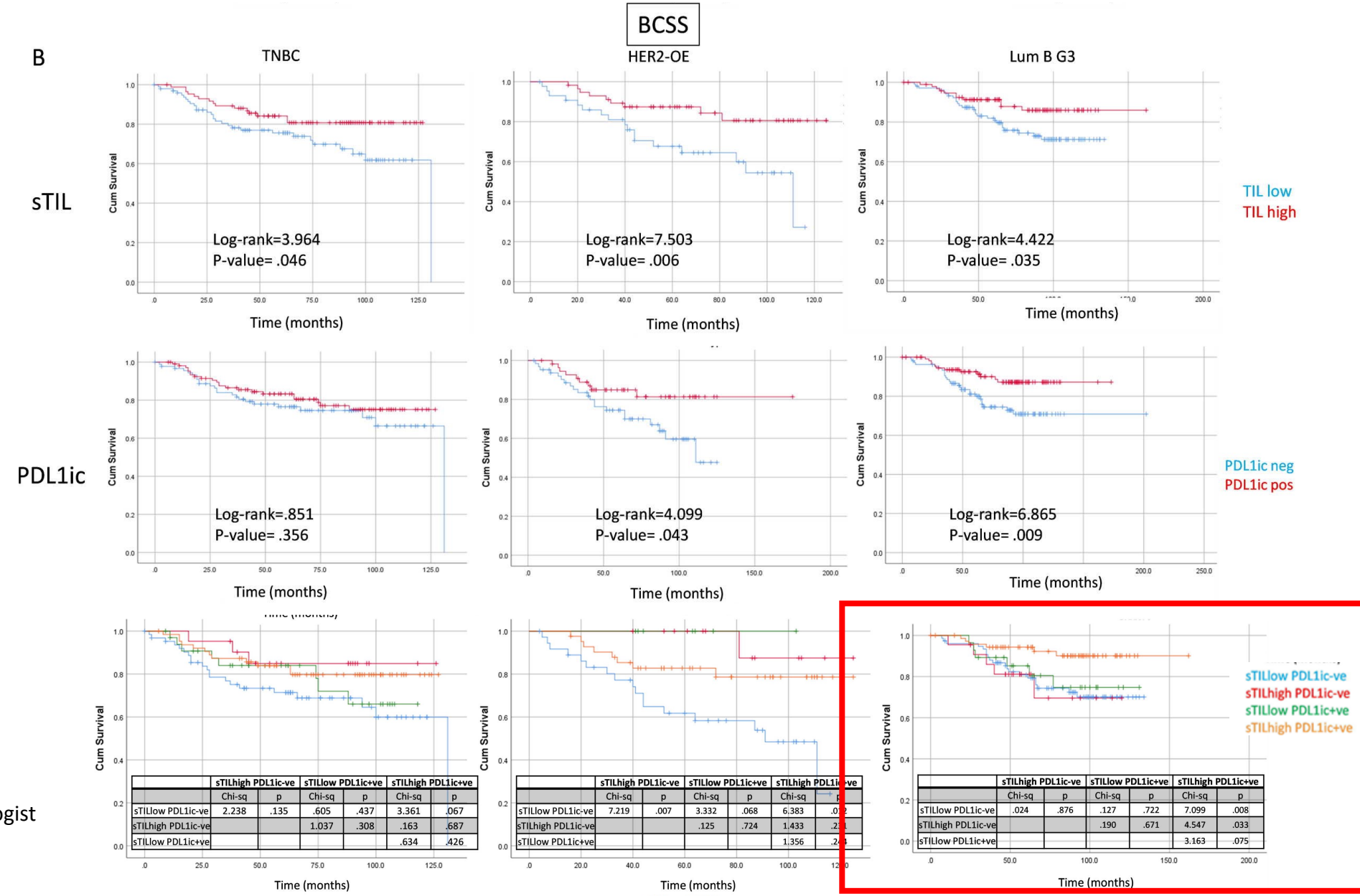
PD-L1 expression on Immune cells in Luminal B G3 tumors

In high–grade luminal B, PD-L1-IC expression was associated positively with high sTIL, HER2, CK5/6, CK14, HVEM, HLAs and PD1+TIL

Ni Y et al 2022 Oncologist

		Overall				LumB G3			HER2-OE			TNBC		
		Neg	Pos	Total	p-value	**Neg**	**Pos**	**p-value**	Neg	Pos	p-value	Neg	Pos	p-value
Grade	1	207	25	232	<.001	-	-	-	1	0	.456	7	0	<.001
	2	551	176	727		-	-		17	12		33	14	
	3	394	399	793		-	-		67	66		79	126	
FF	Absent	823	459	1282	.016	**90**	**121**	**<.001**	63	67	.072	80	101	.390
	Present	317	133	450		**61**	**31**		20	10		39	39	
Necrosis	Absent	934	397	1331	<.001	**108**	**102**	**.396**	47	38	.403	71	65	.005
	Present	190	192	382		**41**	**50**		36	38		46	75	
sTIL	Low	934	397	1331	<.001	**103**	**40**	**<.001**	46	9	<.001	80	43	<.001
	High	185	190	375		**26**	**88**		18	53		24	76	
HER2	Negative	978	435	1413	<.001	**118**	**104**	**.034**	-	-	-	118	139	-
	Positive	167	162	329		**35**	**53**		85	78				
CK5/6	Negative	1024	484	1508	<.001	**145**	**134**	**.008**	65	66	.175	83	74	.004
	Positive	112	112	224		**8**	**22**		18	27		35	66	
CK14	Negative	1092	546	1638	<.001	**149**	**144**	**.031**	84	77	1.00	96	107	.336
	Positive	46	52	98		**3**	**12**		1	1		22	33	
PDL1-T	Negative	1016	517	1533	.011	**131**	**137**	**.779**	67	61	.829	106	117	.185
	Positive	98	75	173		**20**	**19**		16	17		10	19	
PD1 TIL	Negative	677	316	993	<.001	**86**	**72**	**.001**	53	46	.031	68	66	.001
	Positive	13	52	65		**2**	**16**		1	7		0	11	
HVEM	Negative	563	276	839	.001	**70**	**60**	**.002**	25	26	1.00	50	56	.970
	Positive	48	49	97		**1**	**12**		20	19		10	11	
HLA status	All low	378	115	493	<.001	**46**	**22**	**<.001**	29	15	.041	34	20	<.001
	Mixed	205	147	352		**23**	**39**		15	21		23	26	
	All high	68	116	184		**11**	**28**		9	18		9	37	

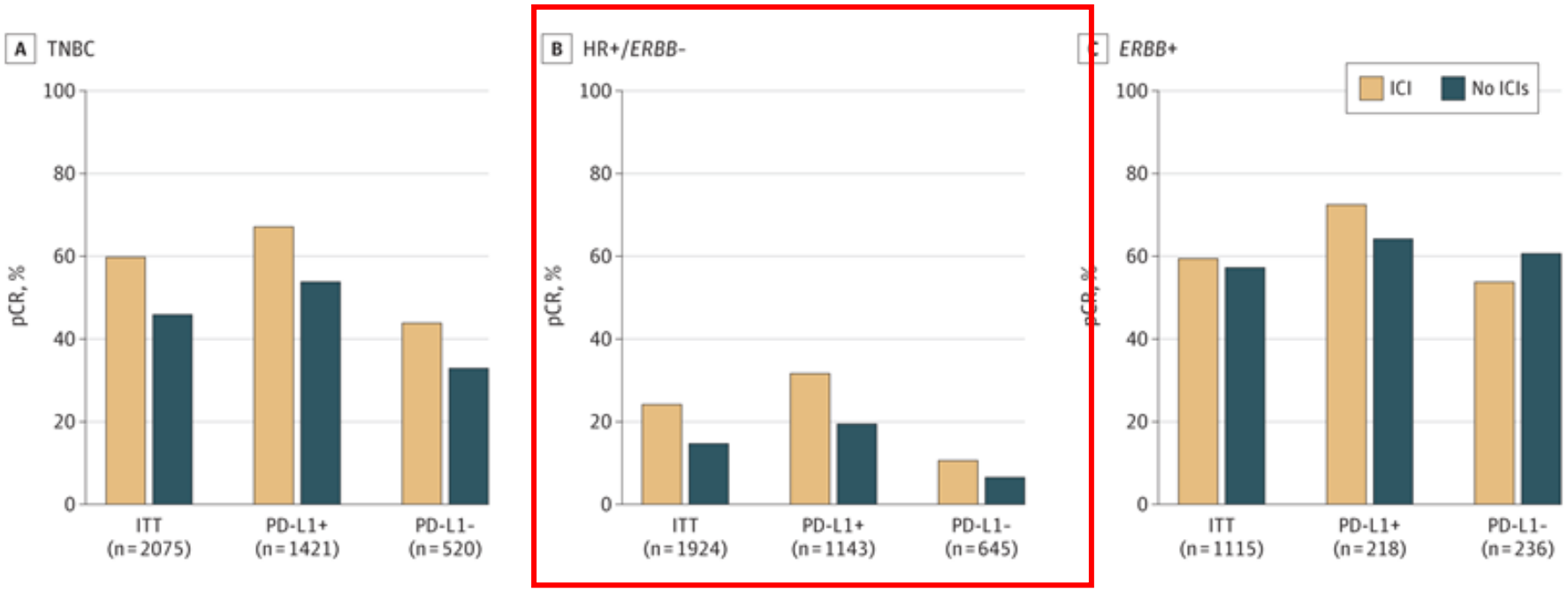
PD-L1 expression on Immune cells and outcome



Ni Y et al 2022 Oncologist

Neoadj ICB plus chemo in early BC (ER neg)

- Addition of ICB therapy – improved pCR rate in TNBC and HR+HER2- tumors, but not HER2+ cancers
- (For TNBC, the benefit is similar in PD-L1 +ve and –ve tumors)
- In HR+/HER2− tumors, adding ICB improved the overall pCR rate from 14.8% to 24.6% (95% CI, 1.49-2.36)
- (Greater benefit in patients with PD-L1+ tumors (pCR rates increased from 19.7% to 31.9% vs 6.8% to 10.9% in PD-L1–negative disease))



Meta-analysis involving 5114 patients from 9 RCTs
TNBC : GeparNuevo 2019; KEYNOTE-522 2020; IMpassion031 2020; NeoTRIP 2022)
HER2+ : IMpassion050 2022; APTneo 2023
ER+(HR+) HER2- : I-SPY2 2020; CheckMate7FL 2023

Villacampa G et al 2024 JAMA Oncol

ER-low validation

Table2. Comparison of the initial and repeated ER immunohistochemical expression

Initial core biopsy	Repeated ER staining			Total
	Negative (<1%)	Low-positive (1%-9%)	Positive (≥10%)	
ER 1%-9%	30 (47%)	16 (25%)	18 (28%)	64
ER 10%	3 (30%)	2 (20%)	5 (50%)	10
ER 11%-30%	11 (38%)	4 (14%)	14 (48%)	29

Table3. Comparison of the initial and repeated ER immunohistochemical expression, RNAscope, and RT-PCR

Initial core biopsy	RNAscope		Total	RT-qPCR		
	Negative	Positive		Negative	Positive	Total
ER 1%-9% and ER-negative on repeat	9 (82%)	2 (18%)	11	9 (82%)	2 (18%)	11
ER 1%-9% and ER-positive on repeat	1 (7%)	14 (93%)	15	4 (36%)	7 (64%)	11
ER 10%-30% and ER-negative on repeat	2 (67%)	1 (33%)	3	3 (100%)	0	3
ER 10%-30% and ER-positive on repeat	0	10 (100%)	10	2 (20%)	8 (80%)	10

- Only ¼ remained as ER-low on repeated IHC
- Cases that were ER-negative following repeat ER staining were PR negative

- RNAscope and RT-qPCR results agreed with restaining

Makhlouf S et al 2023 Mod Pathol

Summary

ER testing

ER low

ER low and therapy

Selected Safety Information for KEYTRUDA (pembrolizumab)

Selected Safety Information for KEYTRUDA (pembrolizumab):
Contraindications: None **Precautions**: •Immune-mediated pneumonitis •Immunemediated colitis •Immune-mediated hepatitis and hepatotoxicity •Immune-mediated endocrinopathies •Immune-mediated nephritis and renal dysfunction •Immune-mediated Dermatologic Adverse Reactions •Other immune-mediated adverse reactions •Infusion-related reactions (including hypersensitivity and anaphylaxis) •Complications of allogeneic HSCT in patients after or prior to treatment with KEYTRUDA treatment •Increased mortality in patients with multiple myeloma when KEYTRUDA is added to a thalidomide analogue and dexamethasone •Embryo-fetal toxicity **Adverse Events**: Most common adverse reactions (reported in ≥20% of patients) were: •Keytruda as a single agent fatigue, musculoskeletal pain, rash, diarrhea, pyrexia, cough, decreased appetite, pruritus, dyspnea, constipation, pain, abdominal pain, nausea and hypothyroidism. •Keytruda in combination with chemotherapy and bevacizumab: peripheral neuropathy, alopecia, anemia, fatigue/asthenia, nausea, neutropenia, diarrhea, hypertension, thrombocytopenia, constipation, arthralgia, vomiting, urinary tract infection, rash, leukopenia, hypothyroidism , and decreased appetite. •Keytruda in combination with axitinib: diarrhea, fatigue/asthenia, hypertension, hepatotoxicity, hypothyroidism, decreased appetite, palmar-plantar erythrodysesthesia, nausea, stomatitis/mucosal inflammation, dysphonia, rash, cough, and constipation •KEYTRUDA in combination with lenvatinib: hypothyroidism, hypertension, fatigue, diarrhea, vomiting, stomatitis, weight loss, abdominal pain, urinary tract infection, proteinuria, constipation, headache, hemorrhagic events, palmar-plantar erythrodysesthesia, dysphonia, rash, hepatotoxicity, and acute kidney injury.

For detailed precautions and adverse events, please consult the full prescribing information.