nature communications

Article

https://doi.org/10.1038/s41467-026-74056-2

Risk stratification and relapse pattern in triple-negative breast cancer with pathological complete response after neoadjuvant treatment: the European GAMBIT real-world study

Received: 15 April 2026

Accepted: 19 May 2026

Published online: 24 June 2026

Check for updates

A list of authors and their affiliations appears at the end of the paper

Pathologic complete response after neoadjuvant treatment is considered a surrogate of cure in triple-negative breast cancer, yet around 10% of patients still relapse. Whether baseline stromal tumor-infiltrating lymphocytes can stratify this residual risk is unknown. Here, we report on GAMBIT, a multicentric real-world retrospective study of 2457 patients with triple-negative breast cancer or estrogen receptor-low disease, HER2-negative, of whom 1192 obtained a pathological complete response and 690 have evaluable tumor infiltrating lymphocytes. Among patients with pathological complete response, clinical nodal status and tumor infiltrating lymphocytes are independently prognostic and patients with clinical node-positive/low-tumor infiltrating lymphocytes tumors experience substantially worse outcomes, with five-year distant relapse-free survival of 83.4% and overall survival of 85.8%. In this high-risk subgroup, five-year cumulative incidence of central nervous system reaches 7.5%, including 6.9% presenting as isolated central nervous system relapse. In this work, we identify a high-risk subgroup despite pathologic complete response and provide a framework supporting risk-adapted trial design incorporating central nervous system-directed strategies.

In triple-negative breast cancer (TNBC), a pathological complete response (pCR) after neoadjuvant therapy (NAT) is strongly associated with improved long-term outcomes at the individual-patient level[1–4]. However, pCR does not guarantee cure[1–4], as up to 10% of patients may still relapse[1].

Despite this contradiction, pCR is widely interpreted as a biological surrogate of cure within current clinical and trial frameworks, serving as the primary decision point for post-NAT treatment allocation[5]. Patients with residual disease (RD) are exclusively eligible for post-NAT treatment intensification[6–8], while patients with pCR are indiscriminately enrolled in de-intensification trials (e.g., OptimICE-pCR; OPT-Pembro)[5,9]. This paradigm assumes risk homogeneity within the pCR population. Yet, in the absence of a framework to identify the minority of patients who remain at risk of relapse despite pCR, this assumption may overlook the very subgroup most likely to benefit from escalation, leading to missed therapeutic opportunities.

To date, only baseline tumor stage (cT)[10,11] and nodal status (cN)[10–12] have shown independent prognostic value after NAT, but these factors alone do not fully explain residual risk after pCR. Stromal tumor-infiltrating lymphocytes (TILs) are a validated prognostic biomarker in TNBC[13–16], strongly associated with pCR rates and improved long-term outcomes in unselected NAT-treated populations[14].

e-mail: mariavittoria.dieci@unipd.it

However, whether TILs retain prognostic value specifically within the pCR subgroup remains unclear, with conflicting results from underpowered studies[14,17].

The timing and preferential sites of relapse in TNBC have been extensively characterized, typically involving early recurrences and visceral sites frequently involving the central nervous system (CNS)[18–20], but whether these patterns apply to the far fewer recurrences occurring among patients with pCR remains unknown.

We therefore conducted a multicentric real-world study designed to investigate (i) whether TILs refine prognosis among TNBC with pCR post NAT and (ii) whether the relapse patterns of patients with pCR differ from those with RD.

Here, we show that, among patients with TNBC or estrogen receptor-low, HER2-negative disease, baseline TILs and cN independently stratify prognosis within the pCR population. Their combination identifies a subgroup of patients with node-positive/low-TILs with substantially elevated residual risk despite pCR, with the CNS emerging as a predominant site of first relapse. These findings challenge the assumption of risk homogeneity within the pCR population and provide a framework to refine post-NAT trial design and surveillance strategies.

Results

Patient population

The subdivision of patients in two cohorts is summarized in Fig. 1. Of 2457 patients with TNBC treated with NAT, 1192 (48.5%) had a pCR (pCR cohort). Patients' characteristics according to pCR or RD are presented in Supplementary Table 1. Compared to RD, patients with pCR were younger, had higher histological grade, and Ki67, while presenting less frequently with cT3-4 disease (17.3% versus 28.8%) and cN^+ status (42.9% versus 49.4%). Neoadjuvant immunotherapy use was more common in the pCR cohort (32.5% versus 13.5%, $p < 0.001$), while more frequently used chemotherapy backbones were: anthracyclines (96.4% versus 93.7%), taxanes (92.0% versus 88.9%), and carboplatin (54.0% versus 32.9%). pCR rates varied markedly across regimens, 39% (545/1390) in patients treated with chemotherapy-only, 51% in patients treated with the addition of carboplatin (260/509) and 69% (387/558) among those treated with immunotherapy.

Within the pCR cohort, TILs were available for 690 (57.9%) patients (pCR-TILs cohort) (Supplementary Note 1). Compared with patients without TILs data, the pCR-TILs cohort had a more advanced cT, less ER-low tumors, and imbalances in receipt of immunotherapy, taxanes, and adjuvant chemotherapy and radiotherapy (Table 1).

Median TILs were 15% (IQR 5-35; Supplementary Fig. 1). TILs were higher in younger patients (<50 years; $p = 0.005$) and varied modestly across different NAT regimens: lower in patients treated with immunotherapy ($p = 0.038$), or those who received taxanes ($p = 0.004$) and carboplatin ($p = 0.013$). No significant associations were observed with other clinicopathological variables (Supplementary Table 2).

Prognostic stratification of patients with pCR

Median follow-up was 3.7 years (95% CI = 3.4-4.0) for the pCR cohort and 3.8 years (95% CI = 3.5-4.3) for the pCR-TILs cohort (Fig. 1). DRFS and OS did not differ between pCR-TILs cohort and TILs not available groups (Supplementary Fig. 2)

In the pCR cohort ($N = 1192$), multivariable models identified cN involvement as the only pathological variable independently associated with worse DRFS and OS (Table 2). For DRFS, cN showed a time-dependent effect: during the first 3 years, patients with cN^+ had nearly a three-fold higher risk of recurrence or death (HR = 2.92, 95% CI = 1.76-4.85, $p < 0.001$). Beyond 3 years, the association was no longer significant. Five-year DRFS and OS rates were inferior in patients with cN^+ ($p < 0.001$ for both endpoints) (Supplementary Fig. 3)

In the pCR-TILs cohort, in addition to cN, TILs were independently prognostic (Table 2): each 5% increase was associated with a 14% lower risk of distant relapse or death (HR = 0.86, 95% CI = 0.82–0.91, $p < 0.001$) and a 16% lower risk of death (HR = 0.84, 95% CI = 0.79–0.90, $p < 0.001$). Adding TILs significantly improved model fit (likelihood-ratio χ^2 (1) = 11.70 for DRFS, 11.17 for OS; both $p < 0.001$; $\Delta AIC = -9.7$ for DRFS; −9.2 for OS), and modestly improved discrimination (C-index = 0.59 to 0.66 for DRFS, 0.65 to 0.72 for OS). Modeling TILs at the pre-defined 30% cut-off yielded similar results,

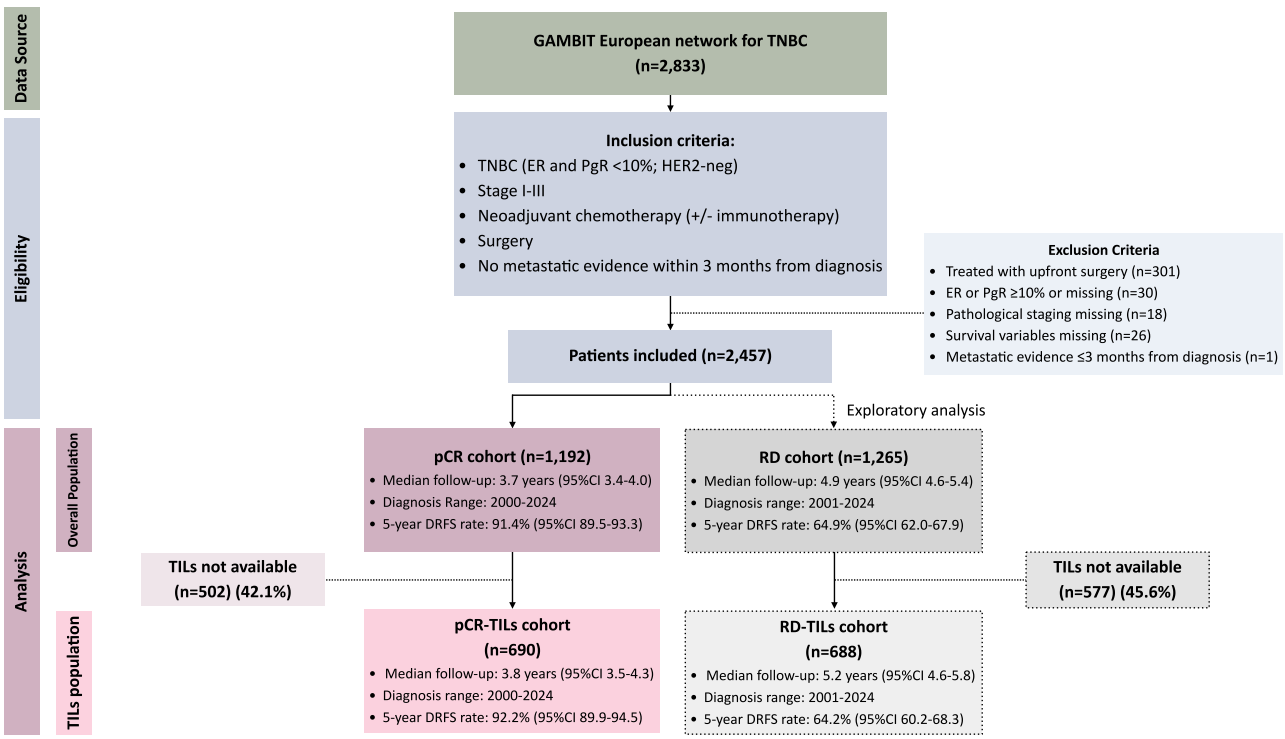

Fig. 1 | Flowchart of patient disposition. Diagram of patient selection and disposition into study cohorts (pCR, RD, and TILs-available cohorts).

https://doi.org/10.1038/s41467-026-74056-2

Table 1 | Clinicopathological characteristics of the pCR cohort (*N* = 1192), according to availability of TILs

Clinicopathological characteristics		pCR Cohort *N* = 1192	pCR-TILs cohort *N* = 690	TILs not available *N* = 502	*p*-value
Age at diagnosis, years	Median (IQR)	49 (41–58)	49 (41–58)	49 (40–59)	0.8
Histology, n(%)	Ductal	1076 (94.6%)	633 (94.9%)	443 (94.1%)	0.4
	Lobular	8 (0.7%)	6 (0.9%)	2 (0.4%)	
	Others	54 (4.7%)	28 (4.2%)	26 (5.5%)	
ER status, n (%	<1	1089 (91.4%)	643 (93.2%)	446 (88.8%)	**0.008**
	≥1-9	103 (8.6%)	47 (6.8%)	56 (11.2%)	
PgR status, n(%)	<1	1131 (94.9%)	660 (95.7%)	471 (93.8%)	0.2
	≥1-9	61 (5.1%)	30 (4.3%)	31 (6.2%)	
HER2 status, n(%)	0	731 (68.7%)	423 (71.8%)	308 (64.8%)	**0.046**
	1+	235 (22.1%)	119 (20.2%)	116 (24.4%)	
	2+/ISH unamplified	98 (9.2%)	47 (8.0%)	51 (10.7%)	
Grade, n(%)	1	1 (0.1%)	0 (0.0%)	1 (0.2%)	0.5
	2	104 (9.4%)	59 (9.1%)	45 (9.7%)	
	3	1006 (90.5%)	589 (90.9%)	417 (90.1%)	
Ki67, %	Median (IQR)	70 (50–80)	70 (50–80)	70 (50–80)	0.2
Clinical Stage, n(%)	I	125 (10.5%)	81 (11.8%)	44 (8.8%)	**<0.001**
	II	852 (71.8%)	460 (66.9%)	392 (78.7%)	
	III	209 (17.6%)	147 (21.4%)	62 (12.4%)	
cT, n(%)	0	6 (0.5%)	1 (0.2%)	5 (1.0%)	**0.001**
	1	215 (18.9%)	132 (20.7%)	83 (16.6%)	
	2	721 (63.3%)	381 (59.6%)	340 (68.0%)	
	3	152 (13.3%)	91 (14.2%)	61 (12.2%)	
	4	45 (4.0%)	34 (5.3%)	11 (2.2%)	
cN, n(%)	Negative	678 (57.1%)	382 (55.5%)	296 (59.2%)	0.2
	Positive	510 (42.9%)	306 (44.5%)	204 (40.8%)	
Neoadjuvant Regimen, n(%)	Chemotherapy	545 (45.7%)	314 (45.5%)	231 (46.0%)	**<0.001**
	Chemotherapy + Carboplatin	260 (21.8%)	191 (27.7%)	69 (13.7%)	
	Chemotherapy + Immunotherapy	387 (32.5%)	185 (26.8%)	202 (40.2%)	
Neoadjuvant chemotherapy backbones, n(%)	Anthracyclines	1146 (96.4%)	660 (95.7%)	486 (97.4%)	0.11
	Taxanes	1092 (92.0%)	606 (87.8%)	486 (97.8%)	**<0.001**
	Carboplatin	643 (54.0%)	374 (54.2%)	269 (53.7%)	>0.9
Adjuvant Chemotherapy, n(%)	No	1088 (94.8%)	593 (91.8%)	495 (98.6%)	**<0.001**
	Yes	60 (5.2%)	53 (8.2%)	7 (1.4%)	
Adjuvant Immunotherapy, n(%)	No	856 (74.8%)	508 (78.6%)	348 (69.9%)	**<0.001**
	Yes	288 (25.2%)	138 (21.4%)	150 (30.1%)	
Adjuvant Radiotherapy, n(%)	No	252 (22.7%)	155 (25.2%)	97 (19.7%)	**0.032**
	Yes	856 (77.3%)	461 (74.8%)	395 (80.3%)	

pCR pathological complete response, *ER* estrogen receptor, *PgR* progesterone receptor, *HER2* human epidermal growth factor receptor 2, *ISH* in situ hybridization, *IQR* interquartile range, *cT* clinical tumor stage, *cN* clinical nodal status, *TILs* tumor-infiltrating lymphocytes. Percentages computed among patients with non-missing data for each variable.
P-values from χ^2 or Fisher's exact tests for categorical variables and Wilcoxon rank sum tests for continuous variables, referring to the comparison between the pCR-TILs cohort and the TILs-not-available cohort. All tests were two-sided. No adjustment for multiple comparisons was made. Bold *p*-values indicate statistical significance ($p < 0.05$).

providing prognostic stratification for DRFS ($p = 0.005$) and OS ($p = 0.010$) (Fig. 2). In multivariable analyses, TILs ≥30% versus <30% were similarly associated with superior outcomes, HR = 0.27 for DRFS (95% CI = 0.15-0.48, $p < 0.001$) and HR = 0.21 for OS (95% CI = 0.10-0.47, $p < 0.001$) (Table 3).

Sensitivity analyses – adjusted for year of diagnosis, using multiple imputation, excluding immunotherapy-treated patients or with ER-low tumors, three institutions contributing either pCR-enriched cases, or lower enrollment rate (Supplementary Note 1) – confirmed the independent prognostic effect of TILs (Supplementary Table 3). Additional exploratory analyses conducted only within immunotherapy-treated patients suggested similar direction of the effect, albeit with wide confidence intervals due to the limited sample size.

We then examined whether the prognostic effect of TILs differed by nodal status. The association was significantly stronger in cN^+ disease (interaction $p = 0.048$ for DRFS; $p = 0.055$ for OS), where each 5% increase in TILs was associated with a 19% reduction in the risk of distant-relapse or death among patients with cN^+ (HR = 0.81, 95% CI = 0.74-0.88; $p < 0.001$), while a weaker effect was observed in cN0 disease (HR = 0.94, 95% CI = 0.85-1.05; $p = 0.3$). A similar pattern was observed for OS (cN^+: HR = 0.75, 95% CI = 0.63-0.89; $p = 0.001$; cN0: HR = 0.98, 95% CI = 0.85-1.14; $p = 0.8$).

Building on the individual prognostic contributions of nodal status and TILs, we examined their joint effect by classifying patients into four strata: cN0/high-TILs (≥30%); cN0/low-TILs (<30%); cN^+/high-TILs; cN^+/low-TILs. The cN^+/low-TILs subgroup, representing 29.4% of the cohort, demonstrated 5-year DRFS 83.4% (95% CI = 77.9-89.4) and OS

https://doi.org/10.1038/s41467-026-74056-2

Table 2 | Multivariable Cox regression analyses for DRFS and OS in the pathological complete response (pCR) cohort and in the pCR-TILs (tumor infiltrating lymphocytes) cohort

			DRFS		OS	
Cohort	**Covariate**		**HR (95% CI)**	***p*-value**	**HR (95% CI)**	***p*-value**
pCR cohort (N = 1192)	**Age (years)**		1.02 (1.00–1.03)	**0.022**	1.02 (1.01–1.04)	**0.002**
	cT	0-2	Ref.		Ref.	
		3-4	1.14 (0.54–2.40)	0.7	1.18 (0.48–2.90)	0.7
	cN	Negative	Ref.		Ref.	
		Positive	2.92 (1.76–4.85)*	**<0.001**	2.84 (1.67–4.81)	**<0.001**
	Grade	2	Ref.		Ref.	
		3	0.59 (0.30–1.14)	0.1	0.83 (0.42–1.65)	0.6
pCR-TILs cohort (*N* = 690)	**TILs (5% increase)**		0.86 (0.82–0.91)	**<0.001**	0.84 (0.79–0.90)	**<0.001**
	Age (years)		1.02 (0.99–1.05)	0.2	1.03 (1.00–1.06)	**0.060**
	cT	0-2	Ref.		Ref.	
		3-4	0.63 (0.28–1.46)	0.3	0.54 (0.20–1.49)	0.2
	cN	Negative	Ref.		Ref.	
		Positive	2.43 (1.20–4.93)	**0.014**	3.47 (1.76–6.85)	**<0.001**
	Grade	2	Ref.		Ref.	
		3	0.85 (0.35–2.07)	0.7	1.60 (0.31–8.11)	0.6

pCR pathological complete response, *cT* clinical tumor stage, *cN* clinical nodal status, *TILs* tumor-infiltrating lymphocytes, *DRFS* distant relapse-free survival, *OS* overall survival, *HR* hazard ratio, *CI* confidence interval, *Ref.* reference category. Analyses are clustered by institution and stratified by neoadjuvant regimen (chemotherapy; chemotherapy including carboplatin; chemotherapy + immunotherapy). *For cN in the DRFS model (pCR cohort), the proportional hazards assumption was violated; a time-varying coefficient was modeled at 3 years. The HR shown is for 0-3 years. HR for DRFS > 3 years: 1.17 (95% CI 0.42-3.32), $p = 0.8$.

Hazard ratios and 95% confidence intervals were estimated using multivariable Cox proportional hazards models with robust sandwich variance estimators clustered by institution and stratified by neoadjuvant regimen. *P*-values were derived from two-sided Wald tests. No adjustment for multiple comparisons was made. No adjustment for multiple comparisons was made Bold values indicate statistical significance (two-sided $p < 0.05$).

85.8% (95% CI = 80.3-91.6) (Fig. 2), while each of the other three subgroups exceeded 95% 5-year rates for DRFS and 97% for OS. Given the near-identical survival distributions of these strata, we pooled them as a comparator: relative to this combined reference, cN$^+$/low-TILs carried approximately 4-fold higher risk of distant relapse (HR = 3.71, 95% CI = 2.05-6.70; $p < 0.001$) and approximately 6-fold higher risk of death (HR = 5.67, 95% CI = 2.90-11.10, $p < 0.001$).

As exploratory analysis, we evaluated the prognostic impact of TILs in the RD cohort. Consistently, patients with low TILs had worse outcomes compared with those with high TILs, and the cN$^+$/low TILs subgroup again emerged as the one with the poorest prognosis compared to other cohorts (Supplementary Fig. 4).

Pattern of relapse in pCR versus RD

Patients with a pCR had a different pattern of relapse compared to those with RD. At 5-years, the cumulative incidence of relapse was lower in the pCR cohort than in RD cohort for any distant relapse (7.2% versus 29.9%, $p < 0.001$), visceral relapse (5.5% versus 20.3%, $p < 0.001$) and any CNS relapse (CNS-any) (3.6% versus 7.0%, $p = 0.001$) (Fig. 3). By contrast, the cumulative incidence of isolated CNS relapse without concurrent extra-CNS disease (CNS-only) was not significantly different between groups (2.6% versus 2.1%; $p = 0.443$) (Fig. 3).

Within the pCR-TILs cohort, relapse risk was largely concentrated in patients with cN$^+$/low-TILs, who had the highest 5-year cumulative incidence of relapse versus the other groups: 13.3% for any distant relapse vs ≤5.0% in other strata (range 1.3-5.0; $p < 0.001$) and 10.3% for visceral relapse versus ≤4.4% in other strata (range 1.3-4.4; $p = 0.002$) (Fig. 3). This difference was particularly evident for CNS endpoints, where the cN$^+$/low-TILs subgroup had 5-year cumulative incidence of 7.5% for CNS-any and 6.9% for CNS-only, whereas all other strata had 5-year cumulative incidence ≤2.8% for CNS-any (range 0–2.8%) and <1% for CNS-only relapse (range 0-0.9%; $p < 0.001$ for both) (Fig. 3). CIFs of relapse in other metastatic sites are provided in Supplementary Fig. 5. Results were consistent in sensitivity analyses excluding patients with ER-low tumors, institutions providing pCR-enriched cases, or with lower enrollment rate (Supplementary Fig. 6)

Among patients with a first distant relapse, the composition of metastatic sites significantly differed by cohort (Supplementary Table 4; Supplementary Fig. 7). In pCR cohort, CNS was the most frequent site (CNS-any 50.7% and CNS-only 37.7%). In the RD cohort, lung (40.7%), lymph nodes (39.0%) and bone (34.0%) predominated, while CNS involvement was less common (CNS-any: 22.7%; CNS-only: 7.0%).

Baseline clinicopathological features and CNS-relapse presentation and management of patients with CNS-any relapse are provided in Supplementary Table 5 and 6, respectively.

Smoothed site-specific hazard functions revealed also distinct temporal dynamics. In the RD cohort, the rates of distant and visceral relapse peaked at 1–2 years after diagnosis, then declined but remained slightly higher than in the pCR cohort up to 5 years (Fig. 4). In the pCR cohort, the risk was lower and transient, peaking at 1–3 years and becoming negligible thereafter. For CNS relapses, the early risk peak was similar in both cohorts, and largely superimposable for CNS-only (Fig. 4). Exploratory analyses stratifying the pCR cohort by cN/TILs risk group showed that the cN$^+$/low-TILs subgroup had an early CNS hazard peak superimposable with that of the RD cohort for CNS-any relapse and numerically higher for CNS-only, whereas CNS relapse risk was near-negligible in all remaining pCR subgroups (Fig. 4).

Discussion

Within a real-world multicentric cohort of patients with TNBC treated with NAT, we demonstrated a significant prognostic heterogeneity among patients with pCR. Baseline TILs and cN independently stratify prognosis in the pCR cohort, and their combination identifies a previously unrecognized subgroup with high residual risk despite pCR. We also observed that CNS emerged as the predominant first site of distant relapse among patients achieving a pCR, presenting frequently as isolated CNS involvement, a pattern largely driven by the high-risk cN$^+$/low TILs subgroup. Together, these findings challenge the long-standing notion that pCR uniformly confers excellent long-term outcomes and provide a framework to refine post-NAT surveillance and trial design for higher-risk pCR subgroups.

https://doi.org/10.1038/s41467-026-74056-2

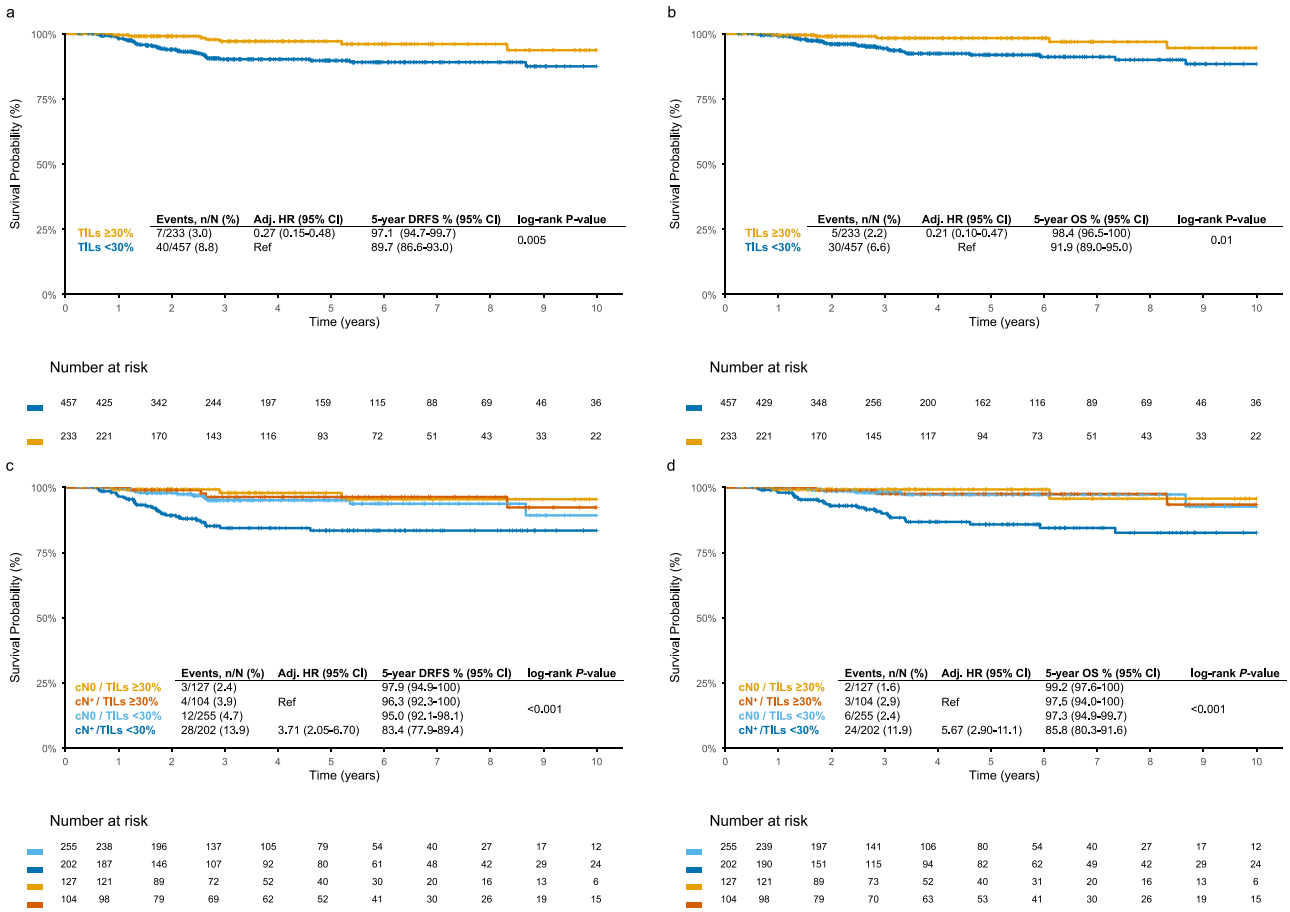

Fig. 2 | Kaplan-Meier survival curves in the pCR-TILs cohort by TILs (gold: ≥ 30%; dark blue: < 30%). DRFS (**a**) and OS (**b**), and combined clinical nodal involvement and TILs (gold: cN0 / TILs ≥ 30%; light blue: cN0 / TILs < 30%; orange: cN+ / TILs ≥ 30%; dark blue: cN+ / TILs < 30%), DRFS (**c**) and OS (**d**). Survival curves were estimated using the Kaplan-Meier method and compared using the log-rank test (two-sided). *p*-values are shown in each panel. Hazard ratios and 95% confidence intervals were estimated using multivariable Cox proportional hazards models with robust sandwich variance estimators clustered by institution and stratified by neoadjuvant regimen. No adjustment for multiple comparisons was made. Source data are provided as a Source Data file.

Table 3 | Multivariable Cox regression analyses for DRFS and OS in the pCR-TILs (tumor infiltrating lymphocytes) cohort with TILs categorized at the pre-defined ≥ 30% cut-off

			DRFS		OS	
Cohort	**Covariate**		**HR (95% CI)**	***p*-value**	**HR (95% CI)**	***p*-value**
pCR-TILs cohort (N = 690)	**TILs**	<30%	Ref.		Ref.	
		≥30%	0.27 (0.15–0.48)	**<0.001**	0.21 (0.10–0.47)	**<0.001**
	Age (years)		1.02 (0.99–1.05)	0.12	1.03 (1.00–1.06)	**0.027**
	cT	0-2	Ref.		Ref.	
		3-4	0.66 (0.28–1.52)	0.3	0.56 (0.20–1.55)	0.3
	cN	Negative	Ref.		Ref.	
		Positive	2.41 (1.18–4.91)	**0.016**	3.45 (1.73–6.90)	**<0.001**
	Grade	2	Ref.		Ref.	
		3	0.89 (0.35–2.26)	0.8	1.68 (0.32–8.73)	0.5

pCR pathological complete response, *cT* clinical tumor stage, *cN* clinical nodal status, *TILs* tumor-infiltrating lymphocytes, *DRFS* distant relapse-free survival, *OS* overall survival, *HR* hazard ratio, *CI* confidence interval, *Ref.* reference category. Analyses are clustered by institution and stratified by neoadjuvant regimen (chemotherapy; chemotherapy including carboplatin; chemotherapy + immunotherapy). All tests are two-sided.

Hazard ratios and 95% confidence intervals were estimated using multivariable Cox proportional hazards models with robust sandwich variance estimators clustered by institution and stratified by neoadjuvant regimen. *P*-values were derived from two-sided Wald tests. No adjustment for multiple comparisons was made. No adjustment for multiple comparisons was made. Bold values indicate statistical significance (two-sided $p < 0.05$).

A key finding is that baseline TILs retain independent prognostic significance even after pCR, revealing immune-related risk heterogeneity after pathological clearance. This contrasts with early reports in which TILs lost prognostic value after pCR[14], and extends pooled analyses in a smaller cohort suggesting TILs-linked survival benefit but without dedicated pCR-specific modeling[17]. Our pCR-restricted, adequately powered analysis, demonstrates that immune contexture remains biologically and clinically relevant, even in patients with pCR.

https://doi.org/10.1038/s41467-026-74056-2

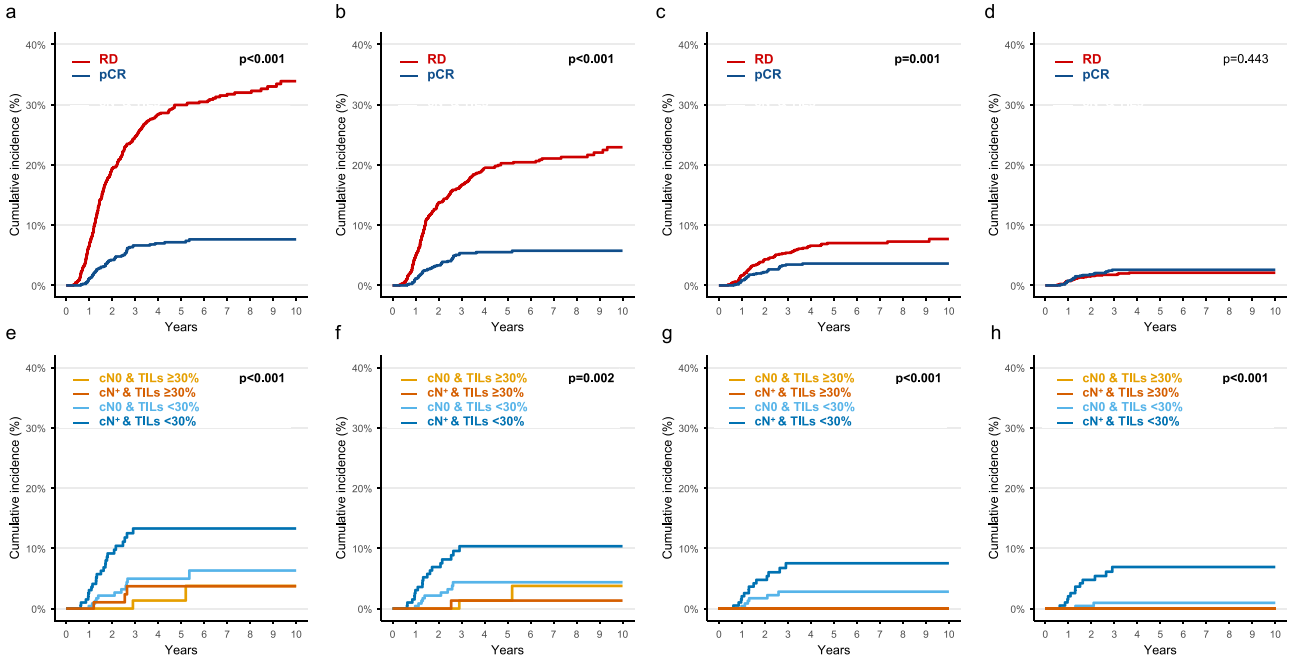

Fig. 3 | Cumulative incidence functions (CIFs) of distant relapse by pathological response and by combined nodal/TILs risk groups. a–d pathological complete response (pCR, blue) versus residual disease (RD, red). **e–h** pCR only, stratified by clinical nodal status (cN0 versus cN+) and baseline tumor-infiltrating lymphocytes (TILs; <30% versus ≥30%): cN0/low-TILs (<30%), cN0/high-TILs (≥30%), cN+/low TILs, cN+/high TILs. Color legends: gold: cN0 / TILs ≥30%; light blue: cN0 / TILs <30%; orange: cN+ / TILs ≥30%; dark blue: cN+ / TILs <30%; Sites: any distant relapse **a**,**e** visceral relapse **b**,**f** CNS-any relapse **c**,**g** CNS-only relapse **d**,**h** CIFs were estimated accounting for competing risks. Differences between groups were tested using Gray's test (two-sided). Bold values indicate statistical significance (two-sided $p < 0.05$). Source data are provided as a Source Data file.

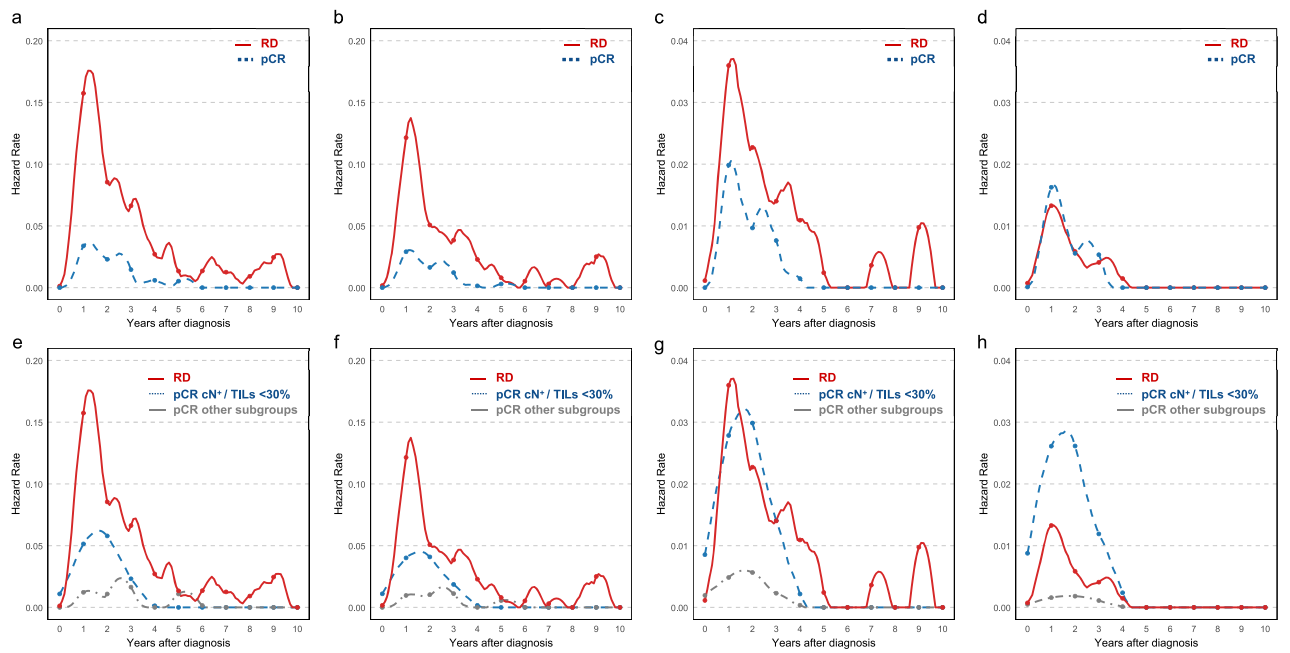

Fig. 4 | Smoothed site-specific hazard functions for first distant relapse in patients with pathological complete response (pCR) versus residual disease (RD). a–d pCR (blue) versus RD (red). **e–h** high-risk pCR (cN-positive/TILs <30%, dark blue), remaining pCR strata (gray), and RD (red). Sites: any distant relapse (**a**,**e**); visceral relapse (**b**, **f**); any CNS relapse with or without extracranial disease (CNS-any; **c**,**g**); isolated CNS relapse without extracranial disease (CNS-only; **d**,**h**). Descriptive, no formal statistical testing was performed. Source data are provided as a Source Data file.

These results have immediate implications for clinical trial design. First, combining nodal status and TILs provides a pragmatic post-pCR stratification tool using standard pathology and staging, identifying patients with cN+/low-TILs as those with a clinically meaningful risk of relapse, with 5-year distant relapse-free survival approximating that of Residual Cancer Burden (RCB) I TNBC following NAT[21], HER2-positive RD cohorts enrolled in escalation trials with Trastuzumab emtansine[22] and, more recently, Trastuzumab-deruxtecan[23]. Second, CNS was a

leading first site of recurrence among patients with a pCR, frequently presenting as isolated CNS relapse. CNS relapse hazard peaked early, within the first 1–2 years, and in the high-risk cN+/low-TILs pCR subgroup closely mirrored the timing and shape observed in RD, whereas the remaining pCR subgroups showed substantially attenuated CNS risk. Similar CNS failure patterns were observed in unselected NAT cohorts[10] and in KEYNOTE-522[24]. Yet post-pCR de-escalation trials[9] do not currently incorporate this risk dimension and those testing escalation strategies, including potentially CNS-active[25–27] antibody drug conjugates[28,29] which currently include only patients with RD. Ultimately, our findings support evaluating CNS-active agents and studying selective CNS surveillance strategies – a topic particularly debated in the metastatic setting[30,31] – in high-risk pCR populations.

Biologically, CNS recurrences despite sensitivity of the primary tumor to systemic treatment may reflect the protective role of the blood-brain barrier as a pharmacologic sanctuary, where limited drug penetration[32–34] permits CNS micrometastatic seeding to persist despite locoregional and extra-CNS disease clearance. This dissociation between primary tumor response and CNS control might be particularly relevant in patients with cN-positive disease and low TILs, in whom CNS hazard dynamics closely mirror those with RD. We hypothesize that in this subgroup, a higher risk for micrometastatic seeding, combined with reduced immunological pressure[35], may impair CNS immunosurveillance, promote clonal heterogeneity[36] and outgrowth of therapy-resistant subclones[37,38]. Future studies should examine whether these mechanisms, pharmacological sanctuary, immune escape, and clonal selection, underlie the observed dissociation between pCR and long-term outcome in this population, and whether they can be therapeutically exploited through CNS-active agents or immune-active strategies.

We acknowledge some limitations. The retrospective multicenter design introduces procedural and treatment heterogeneity, including different pCR rates across regimens; however, all multivariable models were clustered by institution to account for intra-center correlation in outcomes and scoring practices, stratified by treatment regimen to ensure that prognostic estimates reflect within-regimen effects. Sensitivity analyses yielded consistent findings. TILs were scored locally, without central review. This reflects routine clinical practice yet may introduce potential inter-observer variability. However, TILs were evaluated by expert breast pathologists following International TILs Working Group recommendations, analyses were clustered by institution to account for potential systematic between-center differences in scoring practices. We pre-specified analyses at TILs ≥30% cut-off – which has high concordance among readers in expert settings[39] – yielding similar results. A total of 42% of samples within the pCR cohort lacked TILs; tissue availability was driven largely by archival logistics rather than outcomes, thus reducing, but not eliminating the risk of informative missingness. Approximately 30% of patients with pCR received neoadjuvant immunotherapy. While exploratory analyses suggested a similar direction of effect for TILs in this subgroup, the analysis was underpowered, and dedicated validation in larger immunotherapy-treated cohorts with longer follow-up is required. Importantly, immunotherapy does not abolish post-pCR risk, consistent with event rates seen in KEYNOTE-522[40]. Exploratory analyses in RD suggested similar risk stratification by cN and TILs, though interpretation is limited by missing RCB[21] and post-treatment residual TILs[41,42]. Finally, baseline and surveillance CNS imaging were not routinely performed; while this reflects contemporary guidelines[43] and major trial protocols (e.g., KEYNOTE-522[44]), the precise timing of CNS recurrence cannot be inferred, including occult CNS disease at diagnosis.

In conclusion, TILs are independently prognostic in patients with pCR after NAT, and their combined evaluation with cN defines a pragmatic subgroup at elevated risk of relapse and death, with the CNS as a leading first site of relapse. This pragmatic, broadly applicable stratification tool – relying solely on standard hematoxylin and eosin-stained slides, and standard imaging modalities for axillary staging, thus requiring no proprietary technology – demonstrates prognostic heterogeneity within the pCR population and provides a framework to refine post-neoadjuvant trial design, and guide patient allocation in risk-adapted surveillance and escalation trials.

Methods

Ethical considerations

All research described herein complied with all relevant ethical regulations, including the Declaration of Helsinki and applicable data-protection regulations. The present pooled analyses were conducted within the scientific scope of the GAMBIT real-world protocol approved by the coordinating-center Ethics Committee, Comitato Etico Territoriale Area Nord Est Veneto, Padova, Italy (CET ANV 2024-135). Additional de-identified retrospective clinical data from European contributing institutions (Supplementary Note 1) were transferred to the coordinating center under executed Data Transfer Agreements. Written informed consent for the use of clinical data for research was obtained from participants alive at study entry where required by local regulations or committee determinations; when permitted, consent was waived for deceased or lost-to-follow-up patients.

Study design and patient eligibility

This retrospective multicentric, real-world study included patients with stage I-III TNBC (estrogen-receptor [ER] <1% and progesterone-receptor [PgR] <1%, and HER2-negative [immunohistochemistry score 0, or 1+, or 2+ and ISH negative]), or ER-low (ER/PgR 1-9% and HER2-negative) disease. ER-low tumors were included in the GAMBIT study following guidelines recommendations[43], consistent with prior evidence of comparable clinical features[45–47], response to chemotherapy[45,46,48], and, more recently immunotherapy[49–52], as well as similar prognostic significance of obtaining a pCR[46,48]. This is further supported by similar biologic characteristics, such as transcriptomic[45,53,54] and immune profiling[45,53,54], including a similar prognostic effect of immune-infiltration, including TILs[45], as in TNBC[45,54].

Patients were treated at 18 European comprehensive-cancer centers (median year diagnosis 2019, interquartile range [IQR] 2015-2022; range 2000-2024) with any NAT regimen, which was followed by surgery of the primary tumor; any adjuvant treatment was allowed. Case identification was performed locally using institutional clinical databases and pathology records. Three centers contributed a small proportion of pCR-enriched patients (Supplementary Note 1). Staging, treatment and follow-up was performed according to guidelines[43] and local policies; accordingly, CNS imaging was not routinely carried out in asymptomatic patients.

Patients with evidence of metastatic disease within 3 months from primary tumor diagnosis, missing survival variables, pathological staging data, or who did not undergo surgery were excluded.

We categorized patients into two cohorts based on pathological treatment response to NAT, pCR or RD. No matching for baseline characteristics was performed. Patients were further stratified according to the availability of baseline TILs data in pCR-TILs and RD-TILs cohorts (Fig. 1).

Details on data sourcing, collection, are available as Supplementary Note 1.

Pathology

All pathology evaluations were performed locally. pCR was defined as the absence of residual invasive cancer on hematoxylin and eosin (H&E) evaluation of the complete resected breast specimen and all sampled regional lymph nodes following completion of NAT (ypT0/Tis, ypN0). Any residual invasive tumor on resected specimens, both the breast and the axilla, including micrometastatic disease or isolated tumor cells, was classified as RD.

Procedural details for nodal staging and pathologic handling at each institution mandated: (i) axillary ultrasonography at presentation to rule out occult nodal disease, (ii) pre-treatment pathologically-proven confirmation of nodal status involvement by histo/cytological examination in any case with clinically/radiologically suspected lymph node, (iii) all surgically removed lymph nodes post-NAT entirely submitted for histologic evaluation and generally handled according to local policies and international guidelines, resembling the recommendations described by Provenzano et al.[55].

ER and PgR status were assessed on pre-treatment tumor samples by immunohistochemistry (IHC). HER2 status was assessed according to ASCO/CAP recommendations in place at the time of diagnosis.

Baseline stromal TILs were evaluated on treatment-naive H&E-stained slides from the diagnostic core biopsy by expert breast pathologists from 15 institutions (Supplementary Note 1), blinded to patients' outcomes. TILs were recorded as percentage values according to International TILs Working Group recommendations[56], and modeled both as a linear term (per 5-percentage-point increase) and as a binary variable using a predefined ≥30% cut-off. The ≥30% prognostic cut-off for TILs was chosen as previously established in a pooled analysis evaluating TILs in early-stage TNBC treated with chemotherapy[13] and subsequently confirmed in several prognostic studies[13,15,16,57], while showing high concordance among readers (up to 0.93)[58].

Study objectives and sample size

The main objective of this study was to investigate whether baseline TILs are associated with long-term outcomes among TNBC patients with pCR after NAT. The primary population for this analysis was the pCR-TILs cohort (Fig. 1). The primary endpoint, considered for the sample size calculation, was distant relapse-free survival (DRFS). The prespecified primary analysis of the subset of patients achieving pCR used TILs as a continuous variable in a Cox PH regression model on DRFS. Based on pooled literature[13], to detect a HR of 0.83 for each 10% increase in TILs[13] – assuming a standard deviation of approximately 20 percentage points in TILs[13] (equivalent to 4.07 units when TILs are expressed per 5% increments), an event proportion of 10% in the pCR population[1], and a multiple correlation coefficient of $R^2 = 0.20$ between TILs and other covariates – 682 patients provide 80% power at a two-sided significance alpha of 5%[59]. Since our model used TILs expressed per 5% increment, the HR = per 10% increase corresponds to $HR^{1/2} = 0.91$ per 5% increase, and the standard deviation is scaled accordingly to maintain consistency (Supplementary Note 3).

Overall survival (OS) was evaluated as a secondary endpoint. As a preliminary step, we assessed the main prognostic factors in the entire pCR cohort, to frame a reliable benchmark for interpreting the TILs-specific results in the pCR-TILs cohort. The secondary objective was to compare the pattern of relapse in the pCR versus RD cohorts.

The prognostic role of baseline TILs in the RD-TILs cohort was evaluated as an exploratory analysis.

Study reporting

The study is reported in accordance with the ESMO-GROW (European Society for Medical Oncology Guidance for Reporting Oncology real-World Evidence) guidelines for real-world studies[60] and the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines[61]. TILs assessment and reporting follows the REMARK (REporting recommendations for tumor MARKer prognostic studies) guidelines[62] (Supplementary Note 2).

Data sourcing

The 18 contributing institutions, their geographic distribution, enrollment periods, and patient contributions are detailed in Supplementary Note 1. All data were manually derived from electronic health records (EHR) specifically sourced for the present study. Each dataset was obtained from prospectively curated retrospectively captured information on patients with TNBC since local start date at each institution. Core variables include demographics, tumor histology, baseline stromal TIL percentage, clinical tumor and nodal stage, systemic therapy, pCR status, site of first relapse and vital status. Index date for all time-related calculations was the date of histologic diagnosis. Since male breast cancer is exceedingly rare in TNBC, sex was not separately captured in this dataset. Gender was not considered nor collected in the study design.

After executing data-use agreements, centers exported de-identified records using a uniform data dictionary. Data lock was 14 July 2025. A central biostatistician (S.L.) performed logic checks, range checks and duplicate detection. Discrepancies were resolved by site query.

Statistical analyses

Continuous variables were described using medians and interquartile ranges (IQRs), and comparisons between groups were performed using the Wilcoxon rank-sum test. Categorical variables were reported as absolute frequencies and percentages. Group comparisons for categorical variables were conducted using Pearson's χ^2 test, or Fisher's exact test in cases where expected cell counts were less than five.

The distribution of TILs, considered as a continuous variable, was evaluated across categories of baseline clinicopathological characteristics. The Wilcoxon rank-sum test was used for comparisons between two groups, while the Kruskal-Wallis test was applied for comparisons involving more than two groups.

DRFS was defined as the time from diagnosis to distant relapse or death from any cause, OS as the time from diagnosis to death from any cause. Patients who were still alive and had not experienced the event of interest were censored at the date of their last visit or at 10 years of follow-up, whichever occurred first. The median follow-up for DRFS was calculated using the reverse Kaplan-Meier approach. Survival curves were estimated by the Kaplan-Meier method and compared using the log-rank test.

All primary analyses were performed using a complete case approach, excluding individuals with missing data for any covariate included in the models. Multivariable Cox proportional hazards regression models were fitted to identify baseline prognostic factors for DRFS and OS. Models covariates were age (continuous), cT (0-2 versus 3-4), (cN) (negative [cN0] versus positive [cN^+]), histologic grade. In the pCR-TILs cohort, TILs were included in the models both as linear term (5% increments) and as categorical variable (≥30% cut-off). Neoadjuvant regimen (chemotherapy; chemotherapy including carboplatin; chemotherapy plus immunotherapy) was used as a stratification factor. To account for clustering by participating center, all models incorporated a robust (sandwich) variance estimator. Proportional hazards (PH) assumptions were assessed by Schoenfeld residuals[63]. The incremental prognostic value of TILs in the multivariable Cox models was assessed using Harrell's concordance index (C-index), calculated with 1000 bootstrap resamples, alongside the Akaike Information criterion (AIC) and the likelihood ratio test (LRT) for nested model comparison.

No significant non-linearity of TILs was observed using restricted cubic splines (Supplementary Fig. 8). Because nodal status violated the PH assumption in the DRFS model, we fitted a time-dependent Cox proportional hazards model, estimating separate hazard ratios for the 0–3-year and >3-year periods.

Model results were examined in sensitivity analyses: adjusting for (i) year of diagnosis, (ii) performing multiple imputation via chained equations (MICE) for missing baseline covariates in the primary models (TILs were not imputed), (iii) excluding patients treated with immunotherapy, (iv) including only patients treated with immunotherapy, (v) excluding patients with ER-low disease, excluding institutions which (vi) provided pCR-enriched patients, or (vii) enrolled fewer patients per year, with a cut-off identified at six or fewer patients/year.

For the secondary objective, to compare the pattern of first distant relapse between the pCR versus RD cohorts, we collected the type and site of first events classified as: locoregional relapse, distant relapse, or death without prior relapse.

Cumulative incidence functions (CIFs) were estimated non-parametrically using the Aalen-Johansen estimator[64] and compared using Gray's test[65]. Patients without any event were censored at the date of their last visit or at 10 years of follow-up, whichever occurred first. For the analysis of distant relapse as the first event at any site, the event of interest was the first distant relapse, with locoregional relapse (LRR) and death without prior relapse considered competing events. We recorded the sites involved at first distant relapse: CNS, lung, liver, bone, lymph nodes, or other. CNS involvement was classified as "CNS-only" when isolated without extracranial disease, and as "CNS-any" when synchronous with extracranial disease. Sites of first relapse were also grouped as visceral (CNS, lung, liver, other) or non-visceral (bone, lymph nodes). For site-specific cumulative incidence functions (visceral vs non-visceral or by organ site), the event of interest was the first distant relapse involving the site under study. Competing events included LRR, death without prior relapse, and any other first distant relapse not meeting that definition. When locoregional and distant relapses occurred within 2 months of each other, the first event was classified as distant relapse; if they occurred >2 months apart, the earlier event type defined the first event.

For estimation of smoothed site-specific hazard rates, we fitted separate cause-specific Cox models for each site; other first-event types (including death without prior relapse, locoregional relapse, and first distant relapse at other sites) were treated as censored at the time of their occurrence.

Statistical analyses were performed using R programming language (version 4.5.1). A two-sided p-value less than 0.05 was considered statistically significant. When meaningful, p-values were reported to three decimal places, with values smaller than 0.001 reported as $p < 0.001$. Survival analyses were performed with the *survival* and *survminer* packages, while restricted cubic spline modeling was carried out using *rms*. Competing risks analyses were performed with *cmprsk*. Multiple imputation of missing data was conducted using *mice*, and smoothed hazard functions were estimated with *muhaz*. Sample size calculation was implemented via the *powerSurvEpi* R package, for a continuous covariate in a Cox proportional hazards model.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

Source data are provided with this paper. Due to the multicentric and retrospective nature of this pooled analysis, each participating institution retains full ownership of its source dataset and may have institution-specific independent data-sharing procedures. Participants did not provide consent for their data to be shared publicly and for secondary use of data derived from the study without Ethics Committee re-evaluation. De-identified individual patient-level data may be made available to academic researchers for purposes of independent verification or non-commercial methodological research, subject to: (i) submission of a written request specifying the intent and planned analyses; (ii) approval of coordinating center Ethics Committee, relevant local Ethics Committees, (iii) execution of a Data Transfer Agreement (DTA) coordinated through the study sponsor and (iv) compliance with applicable European data protection laws. Data-sharing requests, directed to the study sponsor at Department of Surgery, Oncology and Gastroenterology (DiSCOG), University of Padua (ricerca.discog@unipd.it), will be acknowledged within 60 days. Approved data access will be granted for the duration specified in the relevant DTA. The remaining data are available within the Article, Supplementary Information or Source Data file. Source data are provided with this paper.

Code availability

No custom computer code or mathematical algorithm central to the conclusions of this study was developed. All analyses were performed in R v.4.5.1 using publicly available packages as detailed in Methods.

References

1. Spring, L. M. et al. Pathologic complete response after neoadjuvant chemotherapy and impact on breast cancer recurrence and survival: a comprehensive meta-analysis. *Clin. Cancer Res. J. Am. Assoc. Cancer Res.* **26**, 2838–2848 (2020).
2. Cortazar, P. et al. Pathological complete response and long-term clinical benefit in breast cancer: the CTNeoBC pooled analysis. *Lancet*. **384**, 164–172 (2014).
3. Huang, M. et al. Association of pathologic complete response with long-term survival outcomes in triple-negative breast cancer: a meta-analysis. *Cancer Res.* **80**, 5427–5434 (2020).
4. von Minckwitz, G. et al. Definition and impact of pathologic complete response on prognosis after neoadjuvant chemotherapy in various intrinsic breast cancer subtypes. *J. Clin. Oncol.* **30**, 1796–1804 (2012).
5. Miglietta, F., Dieci, M. V., Griguolo, G. & Guarneri, V. Neoadjuvant approach as a platform for treatment personalization: focus on HER2-positive and triple-negative breast cancer. *Cancer Treat. Rev.* **98**, 102222–102222 (2021).
6. Masuda, N. et al. Adjuvant capecitabine for breast cancer after preoperative chemotherapy. *N. Engl. J. Med.* **376**, 2147–2159 (2017).
7. Tutt, A. N. J. et al. Adjuvant olaparib for patients with *BRCA1* - or *BRCA2* -mutated breast cancer. *N. Engl. J. Med.* **384**, 2394–2405 (2021).
8. Conte, P. F. et al. A-BRAVE trial: a Phase 3 randomized trial with anti-PD-L1 avelumab in high-risk triple-negative early breast cancer patients. *Ann. Oncol.* **0**, 1492–1502 (2025).
9. Valenza, C. et al. Optimizing postneoadjuvant treatment in patients with early breast cancer achieving pathologic complete response. *J. Clin. Oncol.* **42**, 2372–2376 (2024).
10. Boman, C. et al. Prognosis after pathologic complete response to neoadjuvant therapy in early-stage breast cancer: a population-based study. *J. Natl. Compr. Canc. Netw.* **23**, 7093 (2025).
11. Carroll, J. F., Hoskin, T. L., Leon-Ferre, R. A. & Boughey, J. C. Impact of presenting stage on overall survival in patients treated with neoadjuvant chemotherapy for triple negative breast cancer. *Ann. Surg. Oncol.* **31**, 5132–5140 (2024).
12. Huober, J. et al. Identifying breast cancer patients at risk of relapse despite pathological complete response after neoadjuvant therapy. *npj Breast Cancer* **9**, 1–12 (2023).
13. Loi, S. et al. Tumor-infiltrating lymphocytes and prognosis: a pooled individual patient analysis of early-stage triple-negative breast cancers. *J. Clin. Oncol.* **37**, 559–569 (2019).
14. Denkert, C. et al. Tumour-infiltrating lymphocytes and prognosis in different subtypes of breast cancer: a pooled analysis of 3771 patients treated with neoadjuvant therapy. *Lancet. Oncol.* **19**, 40–50 (2018).
15. Park, J. H. et al. Prognostic value of tumor-infiltrating lymphocytes in patients with early-stage triple-negative breast cancers (TNBC) who did not receive adjuvant chemotherapy. *Ann. Oncol.* **30**, 1941–1949 (2019).
16. Leon-Ferre, R. A. et al. Tumor-infiltrating lymphocytes in triple-negative breast cancer. *JAMA* **331**, 1135–1144 (2024).
17. Martín, M. et al. Tumor-infiltrating lymphocytes refine outcomes in triple-negative breast cancer treated with anthracycline-free neoadjuvant chemotherapy. *Clin. Cancer Res.* **30**, 2160–2169 (2024).

18. Dent, R. et al. Triple-negative breast cancer: clinical features and patterns of recurrence. *Clin. Cancer Res.* **13**, 4429–4434 (2007).
19. Kennecke, H. et al. Metastatic behavior of breast cancer subtypes. *J. Clin. Oncol.* **28**, 3271–3277 (2010).
20. van Maaren, M. C. et al. Ten-year recurrence rates for breast cancer subtypes in the Netherlands: a large population-based study. *Int. J. Cancer* **144**, 263–272 (2018).
21. Yau, C. et al. Residual cancer burden after neoadjuvant chemotherapy and long-term survival outcomes in breast cancer: a multicentre pooled analysis of 5161 patients. *Lancet. Oncol.* **23**, 149–160 (2022).
22. Geyer, C. E. et al. Survival with trastuzumab emtansine in residual HER2-positive breast cancer. *N. Engl. J. Med.* **392**, 249–257 (2025).
23. Loibl, S. et al. Trastuzumab deruxtecan in residual HER2-positive early breast cancer. *N. Engl. J. Med.* **394**, 845–857 (2026).
24. Pusztai, L. et al. Event-free survival by residual cancer burden with pembrolizumab in early-stage TNBC: exploratory analysis from KEYNOTE-522. *Ann. Oncol.* **35**, 429–436 (2024).
25. Dannehl, D. et al. The efficacy of sacituzumab govitecan and trastuzumab deruxtecan on stable and active brain metastases in metastatic breast cancer patients—a multicenter real-world analysis. *ESMO Open* **9**, 102995 (2024).
26. Mair, M. J. et al. Understanding the activity of antibody–drug conjugates in primary and secondary brain tumours. *Nat. Rev. Clin. Oncol.* **20**, 372–389 (2023).
27. Tolaney, S. M. et al. ASCENT-05/OptimICE-RD (AFT-65): Phase 3, randomized, open-label study of adjuvant sacituzumab govitecan (SG) + pembrolizumab (pembro) vs pembro ± capecitabine (cape) in patients (pts) with triple-negative breast cancer (TNBC) and residual disease after neoadjuvant therapy (NAT) and surgery. *J. Clin. Oncol.* **41**, TPS619–TPS619 (2023).
28. Bardia, A. et al. TROPION-Breast03: a randomized phase III global trial of datopotamab deruxtecan ± durvalumab in patients with triple-negative breast cancer and residual invasive disease at surgical resection after neoadjuvant therapy. *Ther. Adv. Med. Oncol.* **16**, 17588359241248336 (2024).
29. Dent, R. A. et al. 24TiP A phase III, randomized study of adjuvant sacituzumab tirumotecan (sac-TMT) plus pembrolizumab vs treatment of physician's choice (TPC) in patients with TNBC who received neoadjuvant therapy and did not achieve a pathological complete response (pCR) at surgery. *Ann. Oncol.* **35**, S1413 (2024).
30. Matos, L. et al. Brain imaging screening in metastatic breast cancer: patients' and physicians' perspectives. *Breast* **0**, 104558 (2025).
31. Jerzak, K. J., Warner, E., Knisely, J. P. S. & Sahgal, A. Brain imaging surveillance for patients with metastatic breast cancer: a randomized clinical trial is required to guide practice. *J. Clin. Oncol.* **0**, JCO-25-00356 (2025).
32. Lin, N. U. et al. Sites of distant recurrence and clinical outcomes in patients with metastatic triple-negative breast cancer. *Cancer* **113**, 2638–2645 (2008).
33. Pestalozzi, B. C. et al. Identifying breast cancer patients at risk for Central Nervous System (CNS) metastases in trials of the International Breast Cancer Study Group (IBCSG). *Ann. Oncol.* **17**, 935–944 (2006).
34. Sammons, S. L. et al. Prevalence by therapy line and incidence of breast cancer brain metastases in 18,075 patients. *JNCI J. Natl. Cancer Inst*. https://doi.org/10.1093/jnci/djaf048 (2025).
35. Roerden, M. & Spranger, S. Cancer immune evasion, immunoediting and intratumour heterogeneity. *Nat. Rev. Immunol*. 1–17 https://doi.org/10.1038/s41577-024-01111-8 (2025).
36. Karn, T. et al. Association between genomic metrics and immune infiltration in triple-negative breast cancer. *JAMA Oncol.* **3**, 1707–1711 (2017).
37. Hutchinson, K. E. et al. Comprehensive profiling of poor-risk paired primary and recurrent triple-negative breast cancers reveals immune phenotype shifts. *Clin. Cancer Res.* **26**, 657–668 (2020).
38. Kuett, L. et al. Distant metastases of breast cancer resemble primary tumors in cancer cell composition but differ in immune cell phenotypes. *Cancer Res.* **85**, 15–31 (2024).
39. Denkert, C. et al. Standardized evaluation of tumor-infiltrating lymphocytes in breast cancer: results of the ring studies of the international immuno-oncology biomarker working group. *Mod. Pathol.* **29**, 1155–1164 (2016).
40. Schmid, P. et al. Overall survival with pembrolizumab in early-stage triple-negative breast cancer. *N. Engl. J. Med.* **0**, 1981–1991 (2024).
41. Luen, S. J. et al. Prognostic implications of residual disease tumor-infiltrating lymphocytes and residual cancer burden in triple-negative breast cancer patients after neoadjuvant chemotherapy. *Ann. Oncol.* **30**, 236–242 (2019).
42. Dieci, M. V. 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.* **52**, 16–25 (2018).
43. Loibl, S. et al. Early breast cancer: ESMO clinical practice guideline for diagnosis, treatment and follow-up. *Ann. Oncol.* **35**, 159–182 (2024).
44. Schmid, P. et al. Pembrolizumab for early triple-negative breast cancer. *N. Engl. J. Med.* **382**, 810–821 (2020).
45. Massa, D. et al. Immune and gene-expression profiling in estrogen receptor low and negative early breast cancer. *JNCI J. Natl. Cancer Inst*. **116**, 1914–1927 (2024).
46. Dieci, M. V. et al. Impact of estrogen receptor levels on outcome in non-metastatic triple negative breast cancer patients treated with neoadjuvant/adjuvant chemotherapy. *npj Breast Cancer* **7**, 101 (2021).
47. Yi, M. et al. Which threshold for ER positivity? A retrospective study based on 9639 patients. *Ann. Oncol.* **25**, 1004–1011 (2014).
48. Acs, B. et al. Real-world overall survival and characteristics of patients with ER-zero and ER-low HER2-negative breast cancer treated as triple-negative breast cancer: a Swedish population-based cohort study. *Lancet Reg. Health - Eur.* **40**, 100886 (2024).
49. Cardoso, F. et al. Pembrolizumab and chemotherapy in high-risk, early-stage, ER+/HER2− breast cancer: a randomized phase 3 trial. *Nat. Med.* **31**, 442–448 (2025).
50. Loi, S. et al. Neoadjuvant nivolumab and chemotherapy in early estrogen receptor-positive breast cancer: a randomized phase 3 trial. *Nat. Med.* **31**, 433–441 (2025).
51. Sharma, P. et al. Clinical and biomarker results of neoadjuvant phase II study of pembrolizumab and carboplatin plus docetaxel in triple-negative breast cancer (TNBC) (NeoPACT). *J. Clin. Oncol.* **40**, 513–513 (2022).
52. Cherifi, F. et al. PROMENADE: pembrolizumab for early ER-low/HER2-negative breast cancer, real-world French cohort. *ESMO Open* **10**, 105907 (2025).
53. Voorwerk, L. et al. Immune landscape of breast tumors with low and intermediate estrogen receptor expression. *npj Breast Cancer* **9**, 39 (2023).
54. Kimbung, S. et al. Global transcriptional complexity of estrogen receptor–low positive breast cancers in the prospective Swedish population–based SCAN-B cohort. *Clin. Cancer Res.* **31**, 2695–2709 (2025).
55. Provenzano, E. et al. Standardization of pathologic evaluation and reporting of postneoadjuvant specimens in clinical trials of breast cancer: recommendations from an international working group. *Mod. Pathol.* **28**, 1185–1201 (2015).

56. Salgado, R. et al. The evaluation of tumor-infiltrating lymphocytes (TILS) in breast cancer: Recommendations by an International TILS Working Group 2014. *Ann. Oncol.* **26**, 259–271 (2015).
57. Loi, S. et al. Tumor infiltrating lymphocyte stratification of prognostic staging of early-stage triple negative breast cancer. *Npj Breast Cancer* **8**, 1–4 (2022).
58. Kos, Z. et al. Pitfalls in assessing stromal tumor infiltrating lymphocytes (sTILs) in breast cancer. *npj Breast Cancer* **6**, 1–6 (2020).
59. Hsieh, F. Y. & Lavori, P. W. Sample-size calculations for the Cox proportional hazards regression model with nonbinary covariates. *Control. Clin. Trials* **21**, 552–560 (2000).
60. Castelo-Branco, L. et al. ESMO guidance for reporting oncology real-world evidence (GROW). *Ann. Oncol.* **34**, 1097–1112 (2023).
61. von Elm, E. et al. The strengthening the reporting of observational studies in epidemiology (STROBE) statement: guidelines for reporting observational studies. *Lancet. Lond. Engl.* **370**, 1453–1457 (2007).
62. McShane, L. M. et al. Reporting recommendations for tumour MARKer prognostic studies (REMARK). *Eur. J. Cancer* **41**, 1690–1696 (2005).
63. GRAMBSCH, P. M. & THERNEAU, T. M. Proportional hazards tests and diagnostics based on weighted residuals. *Biometrika* **81**, 515–526 (1994).
64. Aalen, O. O. & Johansen, S. An empirical transition matrix for non-homogeneous Markov chains based on censored observations. *Scand. J. Stat.* **5**, 141–150 (1978).
65. Gray, R. J. A class of K-sample tests for comparing the cumulative incidence of a competing risk. *Ann. Stat.* **16**, 1141–1154 (1988).

Acknowledgements

D.M, S.L and M.V.D had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Author contributions

All authors meet the ICMJE criteria for authorship. Each author made substantial contributions to the conception or design of the work and/or the acquisition, analysis, or interpretation of data; drafted the manuscript or critically revised it for important intellectual content; approved the final version; and agrees to be accountable for all aspects of the work. Specific author contributions (CRediT taxonomy) are detailed below: M.V.D. and D.M conceived and designed the study. D.M. and S.L. curated the data. S.L., M.V.D. and D.M. performed the formal analyses. M.V.D. and V.G. acquired funding. S.L. and D.M. developed the analysis. S.L., D.M. and M.V.D. managed project administration. M.V.D. and V.G. supervised the study. S.L., D.M., and M.V.D. validated the analyses. S.L. and D.M. created the visualizations. D.M., M.V.D. and S.L. wrote the original draft. T.F., S.G., C.D., J.-S.F., E.G., M.K., P.V., W.J., C.V., M.L., F.Patanè, F.Piacentini, E.B., A.Z., D.T., A.B., O.G., A.M., L.S., G.F., M.C., M.Ragazzi, E.L., F.M., G.Bonomi, A.Papakonstantinou, L.N., I.Z., P.N., M.Rotolo, H.H., S.N., A.G.-D.-V., A.V. and A.P.Dei Tos contributed to patient enrollment and local data acquisition (Investigation and Resources). All authors reviewed and edited the manuscript and approved the final version.

Funding

This work was supported by: Fondazione AIRC (Associazione Italiana per la Ricerca sul Cancro) under 5 per mille 2019 - ID22759 program-G.L V.G and IG 27152 to Prof. M.V. Dieci). University of Padova, Department of Surgery, Oncology and Gastroenterology DOR 2021-2023 (to VG, MVD); Ricerca Corrente funding from the Italian Ministry of Health (grant number not applicable). Research at the Netherlands Cancer Institute is supported by institutional grants from the KWF and the Dutch Ministry of Health, Welfare and Sport. A.Papakonstantinou is supported with postdoctoral grant by the Swedish Society for Medical Research; Prof. G.Floris is recipient of post-doctoral clinical mandate sponsored by the Research Foundation Flanders (grant number 1800125N-7006); Prof. C. Desmedt is recipient of a grant from the European Research Council (ERC, FAT-BC 101003153). Ioannis Zerdes (IZ) is supported by the Region Stockholm (clinical postdoctoral appointment, FoUI-977295). The funders did not play a role in the design of the study, the collection, analysis, interpretation of the data, the writing of the manuscript or the decision to submit the manuscript for publication.

Competing interests

All authors reported no conflict of interest (COI) for the present work. Outside the submitted work the following authors reported potential COIs: • D.Massa. received travel/accommodation support from Roche, Lilly. All outside the submitted work. • T.Foukakis: invited Speaker, Payment to Institution: Astra Zeneca, Gilead Sciences, Novartis, Daiichi Sankyo; royalties, personal: Wolters Kluwer (authorship of two chapters in UpToDate); clinical trial support to Institution: Astra Zeneca, Coordinating PI (research grant and study drug), Novartis, Coordinating PI (research grant and study drug), Veracyte, Coordinating PI (discount on the ProsignaPAM50 assay in ARIADNE clinical trial). All outside the submitted work. • I.Zerdes: institutional research grants from Gilead and honoraria paid to his institution from Novartis. • A. Botticelli. had a role in advisory boards and steering committees and has been an invited speaker for Novartis, Roche, Eli Lilly, Pfizer, AstraZeneca, Daiichi Sankyo, Gilead, Merck Sharp & Dohme, Bristol Myers Squibb and Gentili. All outside the submitted work. • A. Zambelli: received honoraria for consultancy and advisory board from Roche, Novartis, Eli Lilly, AstraZeneca, Pfizer, Exact Sciences, Merck, Daiichi Sankyo, Gilead, Seagen, Menarini and Stemline • W. Jacot: reports grants, personal fees and non-financial support from Astra Zeneca, personal fees and non-financial support from Eisai, personal fees and non-financial support from Novartis, personal fees and non-financial support from Roche, personal fees and non-financial support from Pfizer, personal fees and non-financial support from Eli Lilly, personal fees from MSD, personal fees from BMS, personal fees and non-financial support from Chugai, personal fees from Seagen, grants and personal fees from Daiichi Sankyo, personal fees and non-financial support from Gilead. All outside the submitted work. • J.-S.Frenel. has reported a consulting/advisory role for AstraZeneca, GSK, Eisai, MSD, Lilly, Pfizer, Novartis, Daiichi Sankyo, and Seagen, honoraria from AstraZeneca, GSK, Eisai, MSD, Lilly, Pfizer, Novartis, Daiichi Sankyo, and Seagen, support for attending meetings from AstraZeneca, GSK, Eisai, MSD, Lilly, Pfizer, Novartis, Daiichi Sankyo, and Seagen, and participation on advisory boards for AstraZeneca, GSK, Eisai, MSD, Lilly, Pfizer, Novartis, Daiichi Sankyo, and Seagen. All outside of the submitted work • E. Bria: is supported by the Associazione Italiana per la Ricerca sul Cancro (AIRC, Investigator Grant No. IG20583), by Institutional funds of Università Cattolica del Sacro Cuore (UCSC-project D1), and funds of Ministero della Salute (Ricerca Corrente 2024–2025). He has received grants or contracts from Astra-Zeneca, Roche and honoraria for lectures from Merck-Sharp & Dome, Astra-Zeneca, Pfizer, Eli-Lilly, Bristol-Myers Squibb, Novartis, Takeda and Roche; E.B. has been member of Data Safety Monitoring Board or Advisory Board of Merck-Sharp & Dome, Pfizer, Novartis, Bristol-Myers Squibb, Astra-Zeneca, Roche, Amgen and Celltrion. All outside of the submitted work • M. Lambertini: reports advisory role for Roche, Lilly, Novartis, AstraZeneca, Pfizer, Seagen, Gilead, MSD, Pierre Fabre, Menarini, Nordic Pharma and Exact Sciences; receiving speaker honoraria from Roche, Lilly, Novartis, Pfizer, Sandoz, Libbs, Daiichi Sankyo, Takeda, Ipsen, Menarini and AstraZeneca; receiving travel grants from Gilead, Roche, and Daiichi Sankyo; receiving research funding (to his institution) from Gilead; and having nonfinancial interests as a member of the national council of the Italian Association of Medical Oncology (AIOM). All outside of the submitted work. • A.Matikas:. Speaker (paid to institution): Seagen, Roche; Expert consultation (no financial or non-financial compensation): Veracyte, Roche,

AstraZeneca; Advisory Board (no financial or non-financial compensation): Nordic Pharma; Research funding (paid to institution): MSD, AstraZeneca, Novartis, Veracyte. All outside the submitted work. • M.Kok: Received research funding paid to the institute from: BMS, Roche, Astra Zeneca. Advisory roles compensated to the institute for: Astra Zeneca, Daiichi Sankyo, Domain Therapeutics, Alderaan, BMS, MSD, Roche outside the submitted work. Speakers' fee compensated to the institute from Roche, BMS and Gilead. All outside the submitted work. • V.Guarneri receives personal fees for advisory board membership from AstraZeneca, Daiichi Sankyo, Lilly, Gilead Sciences, MSD, Novartis, Pfizer, Olema Oncology, Pierre Fabre, Roche, and Menarini Stemline; as an invited speaker for AstraZeneca, Daiichi Sankyo, Lilly, Exact Sciences, Gilead, GSK, Novartis, Roche, Zentiva, and Menarini Stemline; and for expert testimony from Lilly; and is listed as co-inventor in patent applications for HER2DX. All outside the submitted work. • M.V. Dieci declares (outside the submitted work) personal fees for an advisory/consultancy role from Lilly, Novartis, Pfizer, Roche, Seagen, Gilead Sciences, Exact Sciences, Daiichi Sankyo, AstraZeneca, and Merck Sharpe & Dohme (MSD); research grants to her institution from Roche and AstraZeneca; honoraria for a speaker role from Lilly; travel/accommodation support from Roche, Gilead Sciences, and Lilly; and is listed as co-inventor in patent applications for HER2DX. All outside the submitted work. • P.Vigneri: Advisory role and honoraria from AstraZeneca, Daiichi-Sankyo, Eli Lilly, Gilead, Incyte, Istituto Gentili, Novartis, Pfizer, Roche, Seagen, and Teva. Support for travel and accommodation from Daiichi-Sankyo, Eli Lilly, and Novartis. Research funding (to the institution) from Novartis and AstraZeneca. All outside the submitted work. • F.Martorana: Research funding (to the institution) from Gilead. Fees for lectures from Eli Lilly, Pfizer, Novartis, and Gilead. Travel grants from Eli Lilly, Pfizer, Novartis, and Gilead. All outside the submitted work. • C. Vernieri: reports personal fees from Novartis, Pfizer, Eli Lilly and Company, AstraZeneca, Gilead Sciences, Daiichi Sankyo, MSD, and Menarini Stemline. All outside the submitted work • A. Vingiani reports personal fees from Roche and Illumina outside the submitted work. The other authors declare no conflicts of interest.

Additional information

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41467-026-74056-2.

Correspondence and requests for materials should be addressed to Maria Vittoria Dieci.

Peer review information *Nature Communications* thanks the anonymous reviewers for their contribution to the peer review of this work. A peer review file is available.

Davide Massa[1,2], Theodoros Foukakis[3,4], Sylvie Giacchetti[5,6], Christine Desmedt[7], Jean-Sebastien Frenel[8,9], Elisa Gasparini[10], Marleen Kok[11,12], Paolo Vigneri[13,14], William Jacot[15], Claudio Vernieri[16,17], Matteo Lambertini[18,19], Francesca Patanè[19], Federico Piacentini[20], Emilio Bria[21,22,23], Alberto Zambelli[24,25], Dario Trapani[26,27], Andrea Botticelli[28], Ornella Garrone[29], Stefania Lando[1,30], Alexios Matikas[3,4], Laetitia Someil[5,6], Giuseppe Floris[31], Mario Campone[32], Moira Ragazzi[33,34], Esther Lips[35], Federica Martorana[13,14], Giorgio Bonomi[1,2], Andri Papakonstantinou[3,36], Lorenzo Nicolè[37], Ioannis Zerdes[3,4], Patrick Neven[38], Martina Rotolo[39,40], Hugo Horlings[41], Sabrina Nucera[13,14], Amélie Gudin-De-Vallerin[42], Andrea Vingiani[16], Angelo Paolo Dei Tos[43,44], Valentina Guarneri[1,2], Maria Vittoria Dieci[1,2] ✉, On behalf of the GAMBIT Consortium

[1]Department of Surgery, Oncology and Gastroenterology (DiSCOG), University of Padova, Padova, Italy. [2]Oncology 2, Veneto Institute of Oncology IOV IRCCS, Padova, Italy. [3]Department of Oncology/Pathology, Karolinska Institutet, Stockholm, Sweden. [4]Breast Center, Theme Cancer, Karolinska Comprehensive Cancer Center, Stockholm, Sweden. [5]APHP, Senology Unit, Saint Louis Hospital, Paris, France. [6]Université Paris Cité, Paris, France. [7]Laboratory for Translational Breast Cancer Research, Department of Oncology, KU Leuven,, Leuven, Belgium. [8]Department of Medical Oncology, Institut de Cancerologie de l'Ouest, Saint Herblain, France. [9]Centre de Recherche en Cancérologie et Immunologie Nantes Angers, Nantes Université, Nantes, France. [10]Medical Oncology, Comprehensive Cancer Centre, AUSL-IRCCS of Reggio Emilia, Reggio Emilia, Italy. [11]Division of Tumor Biology and Immunology, The Netherlands Cancer Institute, Amsterdam, the Netherlands. [12]Department of Medical Oncology, The Netherlands Cancer Institute, Amsterdam, the Netherlands. [13]Department of Clinical and Experimental Medicine, University of Catania, Catania, Italy. [14]Medical Oncology Unit, Istituto Clinico Humanitas, Misterbianco, Catania, Italy. [15]Department of Medical Oncology, Institut du Cancer de Montpellier (ICM), Montpellier, France. [16]Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy. [17]IFOM ETS, The AIRC Institute of Molecular Oncology, Milan, Italy. [18]Department of Internal Medicine and Medical Specialties (DiMI), University of Genova, Genova, Italy. [19]Academic Medical Oncology Unit, Ente Ospedaliero Ospedali Galliera, Genova, Italy. [20]Department of Medical and Surgical Sciences for Children and Adults - University Hospital of Modena and Reggio Emilia, Reggio Emilia, Italy. [21]Department of Medical Oncology, Comprehensive Cancer Center, Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Rome, Italy. [22]Università Cattolica del Sacro Cuore, Rome, Italy. [23]Ospedale Isola Tiberina - Gemelli Isola, Rome, Italy. [24]ASST Papa Giovanni XXIII, Bergamo, Italy. [25]School of Medicine and Surgery, Bicocca University,

Milan, Italy. [26]Division of New Drugs and Early Drug Development for Innovative Therapies, European Institute of Oncology (IEO) IRCCS, Milan, Italy. [27]Department of Oncology and Hemato-Oncology, University of Milan, Milan, Italy. [28]Department of Experimental Medicine, Sapienza University of Rome, Rome, Italy. [29]Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy. [30]Unit of Biostatistics, Epidemiology and Public Health, University of Padova, Padova, Italy. [31]Department of Pathology, UZ Leuven, Leuven, Belgium. [32]Institut de Cancérologie de l'Ouest Angers-Nantes, Saint-Herblain, France. [33]Pathology Unit, Azienda USL-IRCCS di Reggio Emilia, Reggio Emilia, Italy. [34]Department of Medical and Surgical Sciences for Children and Adults University of Modena and Reggio Emilia, Modena, Italy. [35]Division of Molecular Pathology, Netherlands Cancer Institute, Amsterdam, The Netherlands. [36]Department of Breast Cancer, Endocrine Tumours, and Sarcoma, Karolinska University Hospital, Stockholm, Sweden. [37]Pathology Unit, Angelo Hospital, Mestre, Italy. [38]Department of Oncology, Gynecologic Oncology, University Hospitals Leuven, Leuven, Belgium. [39]Oncology Unit, Azienda Unità Sanitaria Locale-IRCCS di Reggio Emilia, Reggio Emilia, Italy. [40]University of Modena and Reggio Emilia, Modena, Italy. [41]Department of Pathology, The Netherlands Cancer Institute, Amsterdam, the Netherlands. [42]Department of Pathology, Translational Research Unit, Institut du Cancer de Montpellier, Montpellier, France. [43]Department of Integrated Diagnostics, Azienda Ospedale-Università Padova, Padova, Italy. [44]Department of Medicine, University of Padua, Padua, Italy. *A list of authors and their affiliations appears at the end of the paper. ✉e-mail: mariavittoria.dieci@unipd.it

the GAMBIT Consortium

Davide Massa[1,2], Theodoros Foukakis[3,4], Sylvie Giacchetti[5,6], Christine Desmedt[7], Jean-Sebastien Frenel[8,9], Elisa Gasparini[10], Marleen Kok[11,12], Paolo Vigneri[13,14], William Jacot[15], Claudio Vernieri[16,17], Matteo Lambertini[18,19], Francesca Patanè[19], Giampaolo Bianchini[19], Federico Piacentini[20], Emilio Bria[21,22,23], Alberto Zambelli[24,25], Dario Trapani[26,27], Andrea Botticelli[28], Ornella Garrone[29], Stefania Lando[1,30], Alexios Matikas[3,4], Laetitia Someil[5,6], Giuseppe Floris[31], Mario Campone[32], Moira Ragazzi[33,34], Esther Lips[35], Federica Martorana[13,14], Giorgio Bonomi[1,2], Andri Papakonstantinou[3,36], Lorenzo Nicolè[37], Ioannis Zerdes[3,4], Patrick Neven[38], Martina Rotolo[39,40], Hugo Horlings[41], Sabrina Nucera[13,14], Amélie Gudin-De-Vallerin[42], Andrea Vingiani[16], Angelo Paolo Dei Tos[43,44], Valentina Guarneri[1,2] & Maria Vittoria Dieci[1,2] ✉