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REVIEW

Window of opportunity trials with immune checkpoint inhibitors in triple-negative breast cancer

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Patients with triple-negative breast cancer (TNBC) have a relatively poor clinical outcome. The immune checkpoint inhibitor (ICI) pembrolizumab combined with chemotherapy is the current standard of care in TNBC patients with stage II and III. Monotherapy with ICIs has not been comprehensively assessed in the neoadjuvant setting in TNBC patients, given unfavorable results in metastatic trials. ICIs, however, have been tested in the window of opportunity (WOO) before surgery or standard chemotherapy-based neoadjuvant treatment. The WOO design is well suited to assess an ICI alone or in combination with other ICIs, targeted therapy, radiotherapy or cryotherapy, and measure their pharmacodynamic and clinical effect in this treatment-naive population. Some patients show a good response to ICIs in WOO studies. Biomarkers like tumor-infiltrating lymphocytes, programmed death ligand-1, and interferon-γ signature may predict activity and may identify patients likely to benefit from ICIs. Moreover, an increase in tumor-infiltrating lymphocytes, programmed death ligand-1 expression or T cell receptor expansion following administration of ICIs in the WOO setting could potentially inform of immunotherapy benefit, which would allow tailoring further treatment. This article reviews WOO trials that assessed immunotherapy in the early-stage TNBC population, and how these results could be translated to test de-escalation strategies of neoadjuvant chemotherapy and immunotherapy without compromising a patient's prognosis.

Key words: window of opportunity, immune checkpoint inhibitors, triple-negative breast cancer, tumor-infiltrating lymphocytes, PD-L1

INTRODUCTION

Triple-negative breast cancer (TNBC) is characterized by the absence of estrogen, progesterone, and human epidermal growth factor receptor 2 (HER2) receptors and accounts for ∼15%-20% of breast cancers.[1] TNBC is an aggressive subtype of breast cancer and usually has a poor prognosis. Until a few years ago, the only systemic therapeutic option for patients with TNBC was chemotherapy. The recent approvals of the anti-programmed cell death protein-1 (anti-PD-1) immune checkpoint inhibitor (ICI) pembrolizumab (pembro), poly-ADP ribose polymerase (PARP) inhibitor-based therapy, and the anti-TROP2 antibody-drug conjugate (ADC) sacituzumab govitecan in patients with metastatic TNBC has improved clinical outcomes. Pembro has also been approved in patients with stage II and III TNBC regardless of programmed death ligand-1 (PD-L1) expression level or nodal status, in combination with neoadjuvant chemotherapy (NACT) (KEYNOTE-522),[2] followed by adjuvant pembro [National Comprehensive Cancer Network (NCCN) 2024 guidelines®]. Other options for adjuvant treatment are olaparib for patients harboring germinal *BRCA* mutations (g*BRCA*m) (OLYMPIA[3]) or capecitabine for patients who do not achieve pathological complete response (pCR) (CREATE-X[4]).

ICIs alone have not been adequately assessed in the neoadjuvant setting in patients with TNBC, since monotherapy trials in patients with metastatic disease did not demonstrate an improvement in progression-free survival or overall survival (OS), although there was a trend to better responses in the first line and associated with higher PD-L1

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expression in the tumor biopsies.[5-11] A study has shown that metastatic tumors are immunologically more inert than their corresponding primary tumors except certain gene signatures related to macrophages and angiogenesis that were higher in metastatic lesions.[12] This raises the hypothesis that ICI monotherapy could be better suited in the early TNBC setting. Immunotherapy is relatively well tolerated, especially in comparison to chemotherapy. The specific side-effects of immunotherapy, known as immune-related adverse events (irAEs), are usually managed with corticosteroids or hormone replacement therapy, although 14% of patients may have grade 3 or higher toxicity with some patients requiring hospitalization and/or immunosuppressive treatment.[13] Long-term toxicities of ICIs are an important factor to consider in the benefit-risk assessment of their use in the early disease setting.

Window of opportunity (WOO) studies exploit the 'window' of time after cancer diagnosis and before the initiation of standard treatment. This type of study is mainly used to assess pharmacodynamic biomarkers but also to evaluate early responses to treatment to a short exposure of an investigational agent. The WOO has been employed by some investigators to explore if patients with early-stage TNBC could respond better to immunotherapy alone than those with metastatic disease, without compromising the timely access to surgery and/or standard neoadjuvant therapy. Other less usual WOO trial designs assess the response of new treatments *after* the standard neoadjuvant treatment and *before* the definitive surgery [e.g. BioKey (NCT03197389) and PHOENIX DDR/anti-PD-L1 (NCT03740893)].

The use of one or two doses of anti-programmed cell death protein-1/programmed death ligand-1 [anti-PD-(L)1] in monotherapy or in combination with other treatments like ipilimumab, olaparib, cryotherapy or radiotherapy, has been evaluated in several WOO trials. Although these trials recruited only a small number of patients, they showed that some of them had at least a partial response, and a few even underwent surgery without the need for standard neoadjuvant treatment. A brief course of immunotherapy may allow for immune cell priming which can make subsequent systemic therapy with ICIs and/or chemotherapy more effective. We do not know how the prognosis of patients who responded to one or two doses of immunotherapy in the WOO setting and underwent surgery without neoadjuvant treatment compares to those requiring NACT + pembro, or to the subset of them that also achieves pCR. From a patient's perspective, de-escalation of treatment (both chemotherapy and/or immunotherapy) is an advantage since it reduces side effects and has a shorter treatment duration, thus improving quality of life. Moreover, since pembro has been approved in the early setting regardless of PD-L1 or nodal status,[14] there is a need to find biomarkers that could help identify patients who may benefit from this combination or could potentially need only immunotherapy, a combination of immunotherapy with other targeted agents, or less chemotherapy. This remains to be demonstrated in large randomized trials.

In this article, we discuss the findings from key WOO trials reported to date in TNBC and evaluate the current evidence of the use of immunotherapy alone or with agents other than conventional chemotherapy such as ADCs or PARP inhibitors. We also discuss how the information expected in the future from the ongoing WOO trials in early TNBC could help us to better plan de-escalation of NACT without altering patient's outcomes.

SUMMARY OF WINDOW OF OPPORTUNITY STUDIES WITH IMMUNE CHECKPOINT INHIBITORS FOR TRIPLE-NEGATIVE BREAST CANCER

Several WOO trials using ICIs alone or in combination with other ICIs, immunotherapies different than ICIs, targeted therapies, radiotherapy, cryoablation or novel chemotherapies in TNBC patients (or with a cohort of TNBC) before surgery or standard neoadjuvant treatment have reported results to date. Table 1 summarizes the clinical and translational findings of key trials, and Table 2 lists ongoing trials with no publicly available results yet.

POTENTIAL BENEFITS OF STARTING IMMUNOTHERAPY BEFORE CHEMOTHERAPY

The results reported so far indicate that patients with early TNBC may respond better to ICI monotherapy than patients with metastatic disease, so there is an opportunity to further evaluate the possibility of giving a less toxic therapy and a shorter course of treatment to these patients before surgery. The overall response rate (ORR) to ICI monotherapy was poor in patients with metastatic TNBC. Responses were mainly restricted to a subset of patients with PD-L1+ status and in the first line. In KEYNOTE-012, pembro had 18.5% ORR (PD-L1+)[8]; in KEYNOTE-086, pembro had 5.3% ORR in patients with two or more lines (cohort A)[10] and 21.4% ORR in the first-line setting (cohort B)[11]; in JAVELIN, avelumab had 22.2% ORR (all patients)[7]; and atezolizumab had 24% ORR in the first line and 6% in the second or greater line.[9] The phase III KEYNOTE-119 compared pembro monotherapy versus chemotherapy per physician's choice, and patients with PD-L1+ (≥10%) had 17.7% ORR with pembro versus 9.2% with chemotherapy.[6]

It is well known that some chemotherapy agents such as cyclophosphamide used in the neoadjuvant setting deplete both innate and adaptive immune cells.[43,44] Thus, the administration of chemotherapeutic agents concomitantly with ICIs could theoretically reduce the effect of the immunotherapy to a certain extent, and therefore it could be potentially better to start treatment with an ICI alone in order to expand T cells in certain patients. This effect was observed in the GeparNuevo trial, in which the durvalumab effect on pCR was statistically better in those patients who received immunotherapy before and during NACT.[29] Nonetheless, there was no difference in the long-term outcome (invasive breast cancer-free survival, distant disease-free survival, OS).[32]

Some patients with aggressive tumors, heavy tumor burden, or visceral compromise, however, may require

Table 1. Summary of key window of opportunity (WOO) clinical trials of immune checkpoint inhibitors (ICI) before neoadjuvant therapy or surgery in patients with triple-negative breast cancer (TNBC)

Trial name, phase and NCT	Drug/s assessed in the WOO, number of patients and patient population	Treatment after WOO	pCR and response rate results	Translational results	EFS and OS results	References
KEYNOTE-173 Phase Ib NCT02622074	1× Pembrolizumab (pembro) 3 weeks before NACT with pembro 60 TNBC patients	- Cohort A: (pembro Q3w + nab-paclitaxel w) ×4 → (pembro Q3w + AC Q3w) ×4 - Cohort B and C: (pembro Q3w + Cb Q3w + nab-paclitaxel w) ×4 → (pembro Q3w + AC Q3w) ×4 - Cohort D: (pembroQ3w + Cb w + nab-paclitaxel w) ×4 → (pembro Q3w + AC Q3w) ×4 - Cohort E: (pembrolizumab Q3w + Cb Q3w + paclitaxel w) ×4 → (pembro Q3w + AC Q3w) ×4 - Cohort F: (pembro Q3w + Cb w + paclitaxel w) ×4 → (pembro Q3w + AC Q3w) ×4 Cycle = 3 weeks	pCR: overall 60% - Cohort A: 50% - Cohort B: 80% - Cohort C: 80% - Cohort D: 60% - Cohort E: 20% - Cohort F: 50% CR: - After regimen 1: 10% (E) to 40% (B and D) - After regimen 2: 30% (F) to 60% (B and D)	PD-L1: - Median pre-treatment PD-L1 CPS associated with pCR (30% versus 10%) TILs: - Median pre-treatment sTILs higher for patients with pCR (40% versus 10%) - Median on-treatment sTILs (after cycle 1) higher for patients with pCR (65% versus 25%) - The WOO pembro dose led to a mean absolute increase of sTILs by 11%. This increment was higher in responders than in non-responders: 5% (4%-44%) versus 1% (−3% to 20%) 6-plex markers ($n = 20$): - T cell CD8+/granzyme B+/Ki67+ population correlated with pCR - Myeloid CD163+/MHC-II+ had a negative association with pCR	12-month EFS: - 100% for patients with pCRypT0/Tis ypN0 versus 88% with non-pCR - 98% in patients who received platinum (B-F) versus 80% who did not (A) 12-month OS: - 98% in platinum cohorts (B-F) versus 80% in non-platinum (A) - 80% to 100% across cohorts (100% for four cohorts)	Schmid et al. 2017[15] Schmid et al. 2020[16] Schmid et al. 2021[17]
Ipilimumab + cryoablation Phase I NCT01502592	Arm A: 1× cryoablation (cryo) Arm B: 1× ipilimumab (ipi) Arm C: 1× ipi followed by cryo (ipi + cryo) 19 Patients, all breast cancer subtypes	Surgery	Not reported	Blood markers: - Ipi and ipi + cryo elevated ICOS+ cells and Ki67+ CD4+ and CD8+ T cells at mastectomy, decreased after 30 days but their levels were higher than baseline (more in ipi + cryo, especially in T CD4+ cells) - Increase of IFN-γ with ipi and specially with ipi + cryo - Th1-type cytokines increased over time, especially at 30-days after mastectomy in the ipi + cryo group - No difference in T-cell clonality in the blood of the three groups (but this was observed in the tumor tissue) Tumor markers: - Ki67+ CD4 and Ki67+ CD8 TILs increased in the ipi + cryo group. No differences in	- 1 Patient was confirmed later to be metastatic at the time of the intervention. Remains without evidence of disease after 34 months - Median follow-up 31 months, no events reported	Diab et al. 2014[18] Page et al. 2014[19] Page et al. 2016[20] McArthur et al. 2016[21]

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Table 1. Continued

Trial name, phase and NCT	Drug/s assessed in the WOO, number of patients and patient population	Treatment after WOO	pCR and response rate results	Translational results	EFS and OS results	References
				CTLA-4, PD-1 LAG-3 or TIM-3 on CD4 or CD8 cells - Ipi + cryo caused a decrease in overall TIL number but generated more T-cell clonal expansion		
Pembro/RT Phase I-II NCT03366844	Cohort 1 (high-risk ER+ HER2− breast cancer) Cohort 2 (TNBC) 2 cycles of pembro + 1 RT boost at cycle 2 66 Patients Cycle = 3 weeks	Surgery or NACT followed by surgery	pCR: - 12/20 (60%) patients were ypT0N0, 13/20 (65%) were ypT0/Tis - 15/20 (75%) had RCB 0/1, 4/20 (20%) had RCB 2, and 1/20 (5%) had RCB 3 - 9/11 patients with N+ became ypN0, 1/11 became ypN1mic and 1/11 became ypN1a	TILs: - TIL change after WOO did not correlate with response (but small numbers yet) - TILs ≥10% at baseline correlated to pCR - Patients with RCB 0/1 were strongly associated with CD8+ T cells and lower CD11b+ cells after treatment	Not reported	McArthur et al. 2019[22] McArthur et al. 2021[23]
Camrelizumab/ ablation Phase 2 NCT04805736	Arm 1: microwave ablation Arm 2: Camrelizumab (anti-PD-1) Arm 3: camrelizumab+ microwave ablation 60 Patients, all breast cancer subtypes	Surgery	Not reported	scRNA-seq in the blood (5 patients): - Clonal expansion of CD8+ T cells in the camrelizumab + microwave ablation - Monocytes contributed to enhanced functions of clonal expansion of CD8+ T cells - MHC-I and INF-γ pathways were activated after camrelizumab + microwave ablation	Data not mature	Xie et al. 2023[24] Pan et al. 2024[25]
Decitabine/ pembro Phase II NCT02957968	Cohort A and A2 (TNBC), Cohort B (ER+ HER2−): decitabine one daily dose for 4 days + 1× pembro on day 8 and 22 46 Patients	Standard NACT followed by surgery. Patients on cohort A2 received pembro added to NACT when the approval occurred	pCR: - Cohort A: 50% - Cohort A2: 14.3% - Cohort B: 18.7% - Overall: 32.6%	TILs increase: - Cohort A and A2: from ∼27% to ∼35% - Cohort B: from ∼18% to ∼25% - Overall: from 23.4% to 30.3% PD-L1 increase: - Cohort A and A2: from ∼28% to ∼45% - Cohort B: from ∼12% to ∼30% - Overall: from ∼25% to ∼40% Blood markers: - 59% Decrease in monocytic MDSC ($P < 0.01$) - Decrease of granulocytic MDSC was not significant	Data not mature	Bear et al. 2023[26]

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Table 1. Continued

Trial name, phase and NCT	Drug/s assessed in the WOO, number of patients and patient population	Treatment after WOO	pCR and response rate results	Translational results	EFS and OS results	References
BioKey Phase I NCT03197389	1× pembro at 10 ± 4 days before surgery Cohort A are patients scheduled for upfront surgery (no NACT): - Cohort A1 (TNBC) - Cohort A2 (ER− HER2+) - Cohort A3 (ER+) Cohort B are patients with residual disease after NACT: - Cohort B1 (TNBC) - Cohort B2 (ER− HER2+) - Cohort B3 (ER+) 54 Patients	Surgery	Not assessed	scTCR-seq cohort A: - 9/29 Patients had T-cell clonotype expansion. All had high % of T cells pre-treatment and high PD-L1 expression on T cells - 11/29 Patients with no clonotype expansion had similar % of T cells pre-treatment to patients with clonotype expansion. The rest (9/29) were cold tumors - 61% (range 27%-85%) of expanded T cells were present pre-treatment - T cell expressing PD-1 clonally expanded irrespective of tumor subtype - Expanded CD8+ T cells expressed PRF1, GZMB, CXCL13, HAVCR2, and LAG3 - Expanded CD4+ T cells were T helper 1 expressing IFN-γ and T follicular-helper cells expression BCL6 and CXCR5 markers Baseline biomarkers: - PD-L1+ dendritic cells, CCR2+ or MMP9+ macrophages, or cancer cells expressing MHC-I or MHC-II correlated with T-cell expansion. - By contrast, undifferentiated memory T cells (TCF7+, GZMK+), inhibitory macrophages (CXCR1+, C3+) inversely correlated with the T cell expansion - In TNBC, higher levels of sTILs, PD-1+ sTILs and FOXP3 cells by IHC at diagnosis correlated with expansion of T cells	Not assessed	Bassez et al. 2021[27] Vos et al. 2022[28]
GeparNuevo Phase II NCT02685059	1× Durvalumab/placebo 2 weeks before NACT 174 TNBC patients	(Durvalumab/placebo Q4w + nab-paclitaxel w) ×3 → (durvalumab/placebo Q4w + EC Q2w) ×4 Cycle = 2 weeks	- pCR with durvalumab was 53.4% versus placebo 44.2% (overall response 1.53, $P = 0.182$) - Durvalumab effect on pCR was seen only in the WOO (61.0% versus	PD-L1: - Trend for increased pCR rates in PD-L1-positive tumors, which was significant for PD-L1-tumor cell in durvalumab ($P = 0.045$) and for PD-L1-immune cell in placebo arm ($P = 0.040$)	3-Year iDFS: 85.6% with durvalumab versus 77.2% with placebo (hazard ratio = 0.48, $P = 0.036$) 3-Year DDFS: 91.7% versus 78.4% (hazard ratio 0.31, $P = 0.005$)	Loibl et al .2019[29] Loibl et al. 2019[30] Karn et al. 2020[31] Loibl et al. 2022[32]

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Table 1. Continued

Trial name, phase and NCT	Drug/s assessed in the WOO, number of patients and patient population	Treatment after WOO	pCR and response rate results	Translational results	EFS and OS results	References
			41.4%, overall response 2.22, $P = 0.048$)	TILs: - pCR ($P < 0.01$) was significantly correlated with higher sTILs - iTILs increased in both arms (4.1% placebo, 5.8% durvalumab). iTILs at baseline did not correlate with pCR, but the change did predict pCR only in the durvalumab arm Circulating immune cells: - Macrophages and neutrophils increased, while B, cytotoxic and Th1 cells decreased during treatment (WOO and NACT) - Mast cells at diagnosis associated with pCR - mast cells, Treg and AKT signaling correlated with DDFS Gene expression: - TIL and INF-γ signatures were associated with pCR rate in the whole cohort and in both arms separately TMB: - Patients with pCR had higher TMB at baseline than non-pCR patients	3-Year OS: 95.2% versus 83.5% (hazard ratio 0.24, $P = 0.006$) (independent of pCR) 3-Year iDFS in pCR patients: 95.5% versus 86.1% (hazard ratio 0.22) 3-Year iDFS in non-pCR patients: 76.3% versus 69.7% (hazard ratio 0.67)	Blenman et al. 2022[33] Huebner et al. 2023[34] Virassamy et al. 2023[35]
BELLINI Phase II NCT03815890	Arm 1A (LumB) and 1B (TNBC TILs ≥5%): 2× nivolumab (nivo) Arm 2A (LumB) and 2B (TNBC TILs ≥5%): 2× nivo + 1× ipi Arm 3B (TNBC, TILs ≥50%): 2× nivo + 2× ipi 80 Patients Cycles = 2 weeks	Standard NACT or surgery depending on the response	- 7/31 (23%) Patients had partial response (3 with nivo and 4 nivo/ipi) - 3 Patients had surgery after ICI, 1 with pCR and 1 near-pCR	TILs: - 18/31 Patients (58%) had immune activation, 9 with nivo and 9 nivo/ipi - All patients with a PR had TIL ≥40% - Patients with a PR had higher baseline expression of IFN-γ ($P = 0.014$) - Baseline CD8+ T cells not related to response, however, CD8+ T cells closer to tumor cells were ($P = 0.0014$) Blood: - ctDNA clearance at 4 weeks was evident in 24% of the patients	Data not mature	Nederlof et al. 2022[36]

Continued

Table 1. Continued

Trial name, phase and NCT	Drug/s assessed in the WOO, number of patients and patient population	Treatment after WOO	pCR and response rate results	Translational results	EFS and OS results	References
				- In all patients with a partial response on imaging no ctDNA at +4 weeks or >50% reduction in ctDNA levels		
NeoMono Phase II NCT04770272	Arm A: 1× atezolizumab 2 weeks before NACT Arm B: no WOO, directly to NACT treatment 416 TNBC patients	Arm A and B: (atezolizumab Q3w + Cb w + paclitaxel w) ×4 → (EC Q3w + atezolizumab Q3w) ×4 Cycle = 3 weeks	pCR: - Arm A (WOO): 63.5% - Arm B (w/o WOO): 71.7%	pCR in arm A: - Overall 65.7% - 91.5% in PD-L1 ≥1% - 56.1% in PD-L1 <1% pCR in arm B: - Overall 69% - 82.2% in PD-L1 ≥1% - 64.5% PD-L1 <1% TILs: high TILs at diagnosis or substantial increase after the WOO correlated to pCR Ki67: decrease of Ki67 after WOO treatment correlated to pCR	Data not mature Stopped early at interim because of adapted design	Kolberg-Liedtke et al. 2022[37] Kolberg-Liedtke et al. 2022[38] Erber et al. 2023[39] Kolberg et al. 2023[40]
Olaparib-durvalumab Phase I-II NCT03594396	Olaparib daily for 4 weeks 1× Durvalumab at week 2 54 TNBC patients, including g*BRCA*m or HR-deficiency Cycles = 2 weeks	Standard NACT (4× AC Q3w followed by 4× docetaxel w)	Response rate after 2 weeks of olaparib: - 63.0% (17/27) HR-deficient versus - 25.9% (7/27) HR-proficient tumors (P = 0.006) pCR: - Overall, 75.0% (from 40 out of 54 patients that had surgery at the data cut-off) - In g*BRCA*m, 84.6% (11 out of 13 patients)	Not reported	Data not mature	Im et al. 2022[41]
NeoIRX Phase II NCT04373031	Control arm: pembro on day 1 Arm A: pembro and cyclophosphamide on day 1 + IRX-2 s.c. twice daily for 10 days 12 TNBC patients Cycle: 3 weeks	- Control arm: NACT combined with pembro followed by surgery - Arm A: NACT combined with pembro, with IRX-2 s.c. twice daily for 10 days in between the two NACT regimens, followed by surgery	pCR: - 83% (5/6) versus 33% (2/6) in arm A versus control arm Response rate: 67% (4/6)	TILs: - 67% (4/6) Of the IRX-2 group had brisk increase of TILs after the WOO. All of them (4/4) had pCR after NACT - 2/6 Of the IRX-2 patients had pCR versus 0/6 from the pembro arm after the WOO treatment	Data not mature	Page et al. 2023[42]

AC, anthracycline + cyclophosphamide; Cb, carboplatin; CPS, combined positive score; CR, complete response; ctDNA, circulating tumor DNA; CTLA-4, cytotoxic T lymphocyte associated antigen-4; DDFS, distant disease-free survival; EC, epirubicin + cyclophosphamide; EFS, event-free survival, ER, estrogen receptor; g*BRCA*m, germline *BRCA* mutation; HER2, human epidermal growth factor receptor 2; HLA, human leukocyte antigen; HR, homologous recombination; ICI, immune checkpoint inhibitor; iDFS, invasive disease-free survival; IFN-γ, interferon-γ; ipi, ipilimumab; iTILs, intratumoral tumor infiltrating lymphocytes; LumB, luminal B; MDSC, myeloid-derived suppressor cells; MHC-I, major histocompatibility complex class I; MHC-II, major histocompatibility complex class II; NACT, neoadjuvant chemotherapy; NCT, clinicaltrials.gov number; nivo, nivolumab; OS, overall survival; pCR, pathological complete response; PD-1, programmed cell death protein 1; PD-L1, programmed death ligand-1; pembro, pembrolizumab; PR, partial response; Q2w, every 2 weeks; Q3w, every 3 weeks; Q4w, every 4 weeks; RCB, residual cancer burden; ROR, risk of recurrence; RT, radiotherapy; s.c., subcutaneous; sc-RNA-seq, single-cell RNA sequencing; scTCR-seq, single cell T-cell receptor sequencing; (s)TIL, (stromal) tumor-infiltrating lymphocytes; Th, T helper cells; TMB, tumor mutational burden; TNBC, triple-negative breast cancer; w, weekly; WOO, window of opportunity.

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Table 2. Ongoing WOO trials assessing ICIs or novel immunotherapy agents in TNBC patients

Trial name Phase and NCT	Patient population	Number of patients	Drug/s assessed in the WOO	Treatment after WOO	Endpoints	Estimated completion date
AWARE-1 Phase I NCT04102618	All breast cancer subtypes	26	- Cohort 1 (ER+ HER2−): pelareorep + letrozole - Cohort 2 (ER+HER2−): pelareorep + letrozole + atezolizumab - Cohort 3 (TNBC): pelareorep + atezolizumab - Cohort 4 (ER+ HER2+): pelareorep + trastuzumab + atezolizumab - Cohort 5 (ER− HER2+): pelareorep + trastuzumab + atezolizumab - Cohort 6 (HER2+ irrespective of ER status): pelareorep + trastuzumab	Surgery	Primary: 1) Increase of CelTIL score in all cohorts [time frame: baseline (pre-treatment), day 3, and at surgery (day ∼21)] Key secondary: 1) Increase of CelTIL score in ER+ HER2− and HER2+ [time frame: baseline (pre-treatment), day 3, and at surgery (day ∼21)] 2) Gene expression changes between pre- and post-treatment samples [time frame: throughout]	April 2022 (Early termination when the study met the primary objectives with cohorts 1-4. Only results from cohorts 1 and 2 have been published)
P-RAD Phase II NCT04443348	TNBC and ER+ HER2−	120	- Arm A: no RT + up to 4 × pembro Q3w - Arm B: low-dose RT + up to 4 × pembro Q3w - Arm C: high-dose RT + up to 4 × pembro Q3w	Standard NACT (8 cycles) + up to 4× pembro Q3w (cycles 1-4) followed by surgery. Up to 4× pembro Q3w adjuvant optional	Primary: 1) TILs, CD3+/CD8+ T-cell Breast Immunoscore by immunofluorescence [time frame: 14-21 days after 1st pembro dose] 2) Pathological response in the lymph nodes [time frame: 7 months] Key secondary: 1) Residual cancer burden score [time frame: 24 weeks] 2) pCR [time frame: 24 weeks] 3) Change of TILs and PD-L1 [time frame: 24 weeks] 4) Invasive disease-free survival [time frame: up to 31 months] 5) Event-free survival [time frame: up to 31 months]	December 2023
BreastVAX Phase I-II NCT04454528	TNBC and ER+ HER2−	27	- Arm 1: radiotherapy on day-14 followed by pembro on day-7 - Arm 2: pembro on day-14 followed by radiotherapy on day-7 - Arm 3: pembro on day-14 - Arm 4: no treatment (control arm)	Surgery	Primary: 1) Tolerability of pembro combined with radiation and no excessive delay in surgery [time frame: 2 years] 2) Assess clinical response to treatment based on imaging and histology [time frame: 2 years] Secondary: 1) Changes in the Ki67+ CD8 T cells post treatment in the peripheral blood and in TILs [time frame: 2 years]	August 2024

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Table 2. Continued

Trial name Phase and NCT	Patient population	Number of patients	Drug/s assessed in the WOO	Treatment after WOO	Endpoints	Estimated completion date
Pembro/IORT Phase I NCT02977468	TNBC	15	Pembro at day 1 on 2 cycle + IORT on the surgery day	Surgery	Primary: 1) Increase of TILs [time frame: 3 months]	December 2024
BIS-program Phase II NCT05180006	TNBC and HER2+	210	Cohort 1 (TNBC): - Arm 1A: no treatment - Arm 1B: atezolizumab on day −15 - Arm 1C: atezolizumab on day −15 + ipatasertib daily for 2 weeks starting together - Arm 1D: atezolizumab + bevacizumab on day −15 Cohort 2 (HER2+): - Arm 2A: no treatment - Arm 2B: atezolizumab + trastuzumab + pertuzumab on day −15	Surgery or standard neoadjuvant treatment follow by surgery	Primary: 1) Twofold increase in GzmB+ CD8+ T cell by immunohistochemistry [time frame: 2 weeks] Key secondary: 1) Clinical response [time frame: 2 weeks] 2) pCR rate [time frame: 2 weeks] 3) Changes in CD8+, PD-L1, Ki67 and immune infiltrates [time frame: 2 weeks]	February 2025
IMpALA Phase II NCT04188119	TNBC	42	- Arm A: lansoprazole daily on day 1 + on dose of avelumab after certain time (not specified) - Arm B: lansoprazole and aspirin daily on day 1 + one dose of avelumab after certain time (not specified)	Surgery or standard neoadjuvant treatment followed by surgery	Primary: 1) Mean combined gene expression of COX-2 tumor-promoting genes [time frame: 7 weeks] Key secondary: 1) Post-treatment TILs [time frame: 7 weeks] 2) Mean combined gene expression of the cancer-inhibitory genes in the COX-2 inflammatory signature [time frame: 7 weeks]	May 2025
PHOENIX DDR/ anti-PD-L1 Phase II NCT03740893	TNBC with DDR	81	PART 1: after NACT, preoperative WOO of 2 weeks - Cohort A: standard treatment - Cohort B: ceralasertib twice daily for days 1-14 - Cohort C: olaparib twice daily for days 1-14 - Cohort D: durvalumab on day 1 PART 2: post-operative treatment for 12 months of the same cohorts B-D.	Surgery only for PART 1 No following treatment in PART 2	Primary: 1) Cohort B and C co-primary endpoints: - Change in mean Ki67 after WOO (≥ 33% decrease) [time frame: 2 weeks] - Changes in proliferation 11-gene signature after WOO (≥1.5-fold drop) [time frame: 2 weeks] 2) Cohort D co-primary endpoints: - Change in CD8+ TILs after WOO (≥twofold increase) [time frame: 2 weeks] - Change in IFN-γ+ 4-gene signature after WOO (≥twofold increase) [time frame: 2 weeks] Key secondary: 1) Changes in phosphorylation of ATR and downstream effectors [time frame: 2 weeks] 2) Changes in biomarkers of DDR and adaptive and innate immune response [time frame: 2 weeks]	December 2025

Continued

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Table 2. Continued

Trial name Phase and NCT	Patient population	Number of patients	Drug/s assessed in the WOO	Treatment after WOO	Endpoints	Estimated completion date
					3) Changes in the levels of Th1/IFN-γ response [time frame: 2 weeks] 4) Immune cell population sub-set characterization (T and B cell receptor) [time frame: 2 weeks]	
BRE-03 Phase I NCT04427293	TNBC	12	Lenvatinib daily for 7 days + pembro on day 1	NACT (+/− pembro) followed by surgery, or surgery followed by adjuvant therapy if applicable	Primary: 1) Infiltration of CD8+ TILs (CD45RA-/CD8+/FoxP3-) in primary tumors [time frame: 2 years] Secondary: 1) pCR rate and Ki67 changes [time frame: at surgery] 2) Tolerability assessed by failure to complete NACT [time frame: 30 days after treatment]	July 2026
Nivolumab - Ipilimumab - Cryoablation Phase II NCT03546686	TNBC	80	Ipilimumab + nivolumab followed by cryoablation	Surgery followed by 3× adjuvant nivolumab Q2w	Primary: 1) Event-free survival [time frame: 36 months] Secondary: 1) Invasive disease-free survival [time frame: 36 months] 2) Distant disease-free survival [time frame: 36 months] 3) Overall survival [time frame: 36 months] 4) overall safety [time frame: 36 months]	June 2026
POP-Durva Phase II NCT05215106	TNBC (cT1N0)	200	Durvalumab ×2	Surgery or standard neoadjuvant treatment followed by surgery	Primary: 1) pCR [time frame: day 29 (surgery upfront) or day 22 (patients receiving neoadjuvant treatment] Key secondary: 1) Evaluation of objective response rate [time frame: after 2 administrations of durvalumab]	June 2026

ATR, ataxia telangiectasia mutated and rad3-related; CelTIL, tumor-infiltrating lymphocytes that takes into account the tumor and lymphocyte cellularity; DDR, DNA damage response; ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; IFN-γ, interferon-gamma; IORT, intraoperative radiation therapy; NACT, neoadjuvant chemotherapy; NCT, clinicaltrials.gov number; pCR, pathological complete response; pembro, pembrolizumab; RT, radiotherapy; SBRT, stereotactic body radiation therapy; TILs, tumor-infiltrating lymphocytes; TNBC, triple-negative breast cancer; WOO, window of opportunity.

mmediate treatment with chemotherapy to control tumor growth. Given that immunotherapy could take some time to optimally act on the tumor, ICI monotherapy may not be suitable for such patients who need expeditious treatment. In the BELLINI trial (two doses of nivolumab ± one to two doses of ipilimumab), while 7/31 (23%) patients responded well to the immunotherapeutic drugs, some patients progressed during those 4 weeks of WOO.[36] This highlights the need to use biomarkers that could help to identify patients likely to respond well and thus could benefit from extended ICI in the WOO setting without the need of adding chemotherapy.

For patients in whom additional systemic therapy is needed to control rapid tumor growth, a combination of ICI and targeted agents could be considered where feasible. Of special interest is the combination with PARP inhibitors, which target DNA damage response proteins, but indirectly also promote tumor-infiltrating lymphocytes (TILs) through the up-regulation of chemokines and induction of CD8+ T cells.[45] The use of olaparib for 4 weeks before chemotherapy with a dose of durvalumab in the middle of those 4 weeks significantly improved the pCR rate, which was even higher in patients harboring g*BRCA*m (84.6%, overall 75.0%). There was also a significantly better response to the combination of olaparib and durvalumab in the homologous recombination deficient than in the proficient tumors (63.0% versus 25.9%).[41]

BIOMARKERS AT BASELINE TO PREDICT RESPONSE TO ICIS IN EARLY-STAGE TNBC

In order to de-escalate neoadjuvant treatment, we would need to identify which patients with early-stage TNBC could potentially respond to ICI in monotherapy or in combination. Biomarkers could help us to differentiate these patients from others who may need to start the approved neoadjuvant regimen shortly after diagnosis. Key WOO trials with reported translational and biomarker data are summarized in Table 1.

Tumor-infiltrating lymphocytes

It is well-known by the scientific community that TILs are a prognostic biomarker for early TNBC,[46] and have shown correlation with response to ICIs in several trials. In the KEYNOTE-173, where one dose of single-agent pembro was given before pembro plus NACT, patients with pCR had higher median pre-treatment stromal TILs (sTILs) (40% versus 10%).[16] In the corresponding phase III trial, the KEYNOTE-522, which led to the approval of the combination (but did not have a WOO phase), the relationship between TILs and pCR or event-free survival (EFS) was not assessed. This was done in the phase II NeoPACT trial, which showed a pCR rate of 74% in patients with sTILs ≥30% with shorter treatment than KEYNOTE-522 (six cycles × carboplatin plus docetaxel plus pembro).[47,48]

In the BELLINI trial, all patients with partial response had ≥40% TILs at baseline. Of interest, an in-depth analysis of the population of CD8+ T cells showed no correlation between baseline levels and response; however, when these CD8+ T cells were physically closer to the tumor on spacial analysis, such correlation was observed.[36] In the GeparNuevo (one durvalumab dose) and NeoMono (one atezolizumab dose) WOO trials, higher levels of TILs at baseline were significantly associated with pCR.[29,39] In the WOO trial assessing the combination of pembro + radiotherapy, patients with TILs ≥10% at baseline correlated with a higher rate of pCR.[23] TILs (or CD8+ T cells) evaluation and their correlation with treatment response are part of the endpoints of most of the ongoing WOO trials listed in Table 2.

Programmed death ligand-1

It is reasonable to hypothesize that PD-L1 is a predictor of response to immunotherapy. Several WOO trials have shown a correlation between PD-L1 expression level and pCR. In the GeparNuevo study, a trend for increased pCR rates was observed in PD-L1+ tumors. In the durvalumab arm, there was a significant correlation with PD-L1+ tumor cells; in the placebo arm, however, the correlation was with PD-L1+ immune cells.[29] The NeoMono trial studied the relationship between PD-L1 positivity and pCR. Within the atezolizumab arm, 91.5% of PD-L1+ (≥1%) patients achieved pCR in comparison to 56.1% of the patients with PD-L1−. In the placebo arm the difference was smaller, where 82.2% patients with PD-L1+ achieved pCR vs. 64.5% PD-L1− patients.[39] In the KEYNOTE-173 study, a higher median baseline PD-L1 expression level was observed in tumors with pCR than the ones with no pCR (30% versus 10%).[16,17] In the phase III KEYNOTE-522 and phase II NeoPACT studies, however, PD-L1 failed to predict the response to immunotherapy, although pembro was combined upfront with chemotherapy in contrast to most WOO studies discussed in this review.[2,47]

Interferon-γ signature

Given that the presence of T cells in the tumor correlates with an increased level of interferon-γ (IFN-γ)-related genes, the IFN-γ signature is a good candidate to predict response to the ICIs. In the BELLINI trial, patients with partial response had higher baseline expression of IFN-γ in the tumor.[36] In the GeparNuevo trial, there was a correlation between IFN-γ signature expression with pCR in the whole cohort and in both arms separately.[30,31]

Other biomarkers

The BioKey trial carried out a comprehensive analysis on biopsies (before and after one dose of pembro) using single-cell sequencing. They observed that PD-L1+ dendritic cells, macrophages expressing CCR2 or MMP9, or cancer cells expressing major histocompatibility complex class I (MHC-I) or class II (MHC-II) correlated with T-cell expansion. Undifferentiated memory T cells (TCF7+, GZMK+) and inhibitory macrophages (CXCR1+, C3+), however, were inversely correlated with T-cell expansion.[27]

Other biomarkers have been evaluated in the exploratory analysis of different WOO trials. In KEYNOTE-173, the CD8+/granzyme B+/Ki67+ T-cell population in the tumor showed a positive correlation with a higher pCR rate, while the CD163+/MHC-II+ myeloid population demonstrated a negative correlation.[17] In the GeparNuevo study, higher levels of CCL3 in the blood correlated with a lower rate of pCR in the durvalumab arm.[34]

PCR MAY NOT BE THE BEST SURROGATE BIOMARKER TO ASSESS THE LONG-TERM BENEFIT OF IMMUNOTHERAPY

Achieving pCR is objective evidence of response to therapy; however, some patients who benefit from ICIs do not achieve complete response. In recent years, regulatory agencies have not relied on pCR as a surrogate biomarker for EFS and OS, and often require long-term outcome data before approving a new regimen. In a recent exploratory analysis of KEYNOTE-522, 5-year EFS rates in the pembro and placebo groups were 92.2% versus 88.2% in patients with a pCR, and 62.6% versus 52.3% in the ones without a pCR.[49] The investigators recently announced that the study also met its OS endpoint; it would be interesting to see the difference between pCR and non-pCR patients in both arms as well.[50] Other phase II trials with pembro, such as I-SPY (KEYNOTE-522 regimen) and NeoPACT (pembro + carboplatin + docetaxel), had similar results.[47,51]

The 3-year OS in the GeparNuevo study (which included one dose of atezolizumab in the WOO before atezolizumab + NACT) was 95.2% with durvalumab versus 83.5% with placebo (HR 0.24, $P = 0.006$), regardless of pCR. The phase II NeoTRIP study tested the combination of atezolizumab with carboplatin and nab-paclitaxel followed by anthracyclines, which did not observe any statistically significant improvement in pCR (48.6% versus 44.4%).[52] This contrasts with the positive results of the phase III Impassion-031 (testing the same combination), in which pCR rates were 58% versus 41% in all patients, and 69% versus 49% in the PD-L1+ population.[53] The 2-year EFS was 85% versus 80% and the 2-year OS was 95% versus 90%.[53] GeparDouze/NSABP B-59, which is testing atezolizumab with a similar design to KEYNOTE-522 in a larger population than Impassion-031, will be the confirmatory study for this drug in the (neo)adjuvant setting.[54]

DYNAMIC BIOMARKER CHANGES TO ASSESS THE BENEFIT OF ICIS IN THE WOO

The trials that combined immunotherapy with the neoadjuvant treatment discussed in the previous section show that not all patients with pCR have long-term benefit, and conversely there are some benefits of immunotherapy even in patients who do not achieve pCR. Hence it is necessary to evaluate other surrogate biomarkers that may correlate better with the long-term effect of immunotherapy. Unfortunately, many of the WOO trials discussed in this review do not have data on long-term follow-up yet, so the evaluation of the biomarkers has been done with pCR as evidence of response.

Dynamic increase of TILs

An increase of TILs from baseline to post-WOO treatment has been shown to correlate with pCR. In the KEYNOTE-173 study, where most patients had an increase in sTILs after one dose of pembro, those who had pCR had higher post-WOO sTILs (65% versus 25%).[16] In the GeparNuevo trial, investigators observed an increase of sTILs and an infiltration of sTILs into the tumor nests, resulting in an increase of intratumoral TILs (iTILs). This increase of iTILs following durvalumab monotherapy, but not the baseline levels of iTIL, predicted pCR.[31]

NeoIRX, a WOO trial that assessed the multi-cytokine targeting immunotherapy IRX-2 given with cyclophosphamide and pembro, and also as monotherapy after paclitaxel and before anthracyclines during NACT, has reported initial results in six patients. Four out of those six patients had an increase of TILs and achieved pCR; two of these complete responses were after the WOO treatment only.[42] The ipilimumab + cryotherapy WOO study evaluated TILs in more detail and reported an increase of Ki67+ CD4 and CD8 cells after ipilimumab alone or combined with cryoablation in blood and tumor tissue, but correlation with pCR was not observed.[21] This was also the case in the pembro/decitabine trial, where there was an increase of TILs of ∼7% in each arm, but the correlation with pCR was not observed.[26]

In the BioKey trial, investigators observed that PD-1-expressing T cells were the only ones expanding after the pembro dose, regardless of breast cancer subtype (all were included). Specifically, expanded CD8+ T cells expressed the cytotoxic markers PRF1 and GZMB, the immune cell homing CXCL13, and the exhaustion makers HAVCR2 and LAG3; expanded CD4+ T cells were T helper 1 expressing IFN-γ and T follicular helper cells expressing BCL6 and CXCR5 markers.[27]

An increase of TILs seems to provide clear evidence that ICIs are reactivating and expanding the immune system of the patient. It is currently the primary endpoint in some ongoing trials (displacing pCR to secondary endpoint in some of them). It has emerged as a strong candidate predictive biomarker to evaluate if the patient has benefited from immunotherapy and could be also used to decide if additional cycles of ICIs would be worthwhile when trying to deescalate chemotherapy. It is also affordable and there are robust international guidelines already published and being used on how to score TILs in breast cancer.[55]

T-cell receptor clonal expansion

T-cell receptor (TCR) clonal expansion is another biomarker that indicates immune activation in the patient following immunotherapy. The higher the clonality is, the higher number of different neoantigens the immune system is recognizing in these patients, and therefore, the better the tumor heterogeneity could be addressed. In the ipilimumab + cryotherapy trial, the combination resulted in higher clonal expansion of T cells in the tumor tissue than ipilimumab or cryotherapy alone. This expansion was more difficult to quantify in blood.[21] In the camrelizumab +

microwave ablation WOO study, the investigators observed a clonal expansion of CD8+ T cells in the blood with the combination, in which monocytes contributed to enhanced functions of the clonal expansion.[24] Clonal expansion of T cells has been correlated with ICI response in lung cancer and melanoma.[56,57] This assay is not well standardized, however, and is more costly.

The BioKey trial has extensively studied the TCR expansion provoked by a single dose of pembro. A total of 9 out of 29 patients (31%) had T-cell clonotype expansion. All these patients had a high number of T cells pre-treatment and high PD-L1 expression on those T cells. Some 61% (range 27%-85%) of the expanded T cells were present pre-treatment. A total of 11/29 (38%) patients with no clonotype expansion had similar numbers of T cells pre-treatment to the patients who experienced clonotype expansion. The rest of the patients (9/29, 31%) were cold tumors and did not demonstrate clonotype expansion.[27]

Circulating tumor DNA clearance

Circulating tumor DNA (ctDNA) is a convenient blood biomarker to monitor the response of treatments at different timepoints. In the BELLINI trial, 83% (25/30) of the patients were ctDNA+ at baseline, of whom 24% (6/25) showed clearance after 4 weeks of receiving the WOO treatment (nivolumab or nivolumab + ipilimumab).[36] ctDNA clearance is a good indicator that the tumor growth is being controlled and is not shedding more DNA into the blood. Indeed, ctDNA has been proposed to be added to the TNM (tumor—node—metastasis) staging system by adding a 'B' representing blood in the proposed expanded TNM(B) notation system.[58]

Changes in other biomarkers

Candidate biomarkers reflecting response to treatment are under evaluation. The ipilimumab + cryotherapy trial reported an increase of IFN-γ, ICOS+, and Ki67+ T CD4 and CD8 cells in the blood of both ipilimumab and ipilimumab + cryotherapy cohorts, which decreased after 30 days after mastectomy but maintained a higher level than baseline specially in the ipilimumab + cryotherapy cohort. This trial also showed a profound increase of Th1-type over time in the ipilimumab + cryotherapy arm, and a modest increase of IL-2 and IL-12.[21] In the NeoMono trial, a decrease of Ki67 in the tumor cells after the WOO treatment correlated with pCR.[37] The pembro/decitabine trial reported an increase of PD-L1 expression in the tumor, and a 59% decrease in monocytic myeloid-derived suppressor cells.[26]

DE-ESCALATION OF CHEMOTHERAPY WITH THE USE OF WOO IMMUNOTHERAPY AND OTHER THERAPIES

Some patients may respond exceptionally well to a few doses of one or a combination of two ICIs in the WOO setting, and perhaps may not need further standard neoadjuvant treatment. In the BELLINI trial, three patients underwent surgery after receiving only two doses of nivolumab ± one dose of ipilimumab (not specified); of these, one had pCR and another had a 'near pCR'.[36] New ICIs targeting TIGIT, TIM3, B7.H4, LAG3 or OX40 could potentially have additive or synergistic benefits to currently approved ICIs. Other targeted or immune therapies could be combined to increase response rates; this would likely depend on the biomarker profile.

A proportion of patients who are administered ICIs in the WOO setting who do not have adequate tumor shrinkage, or in whom biomarkers like TILs or PD-L1 do not show a temporal increase, would require treatment with standard NACT. Nonetheless, it may still be possible to de-escalate some chemotherapy doses in cases where a good response occurs early during NACT, and in these carefully selected patients, it could be possible to administer a less toxic and shorter cytotoxic treatment. Taxanes and carboplatin have been shown to be especially effective in TNBC,[59-61] and therefore efforts have been made to de-escalate anthracyclines, which have a less favorable toxicity profile. This question is being prospectively addressed in the SCARLET/SWOG 2212 (NCT05929768) study, which is comparing an 18 weeks of anthracycline-free chemo-immunotherapy neoadjuvant combination (Neo-PACT regimen, pembro + carboplatin + docetaxel)[47] with 24 weeks of the current approved standard therapy (KEYNOTE-522 regimen, pembro backbone plus carboplatin + paclitaxel → doxorubicin/epirubicin + cyclophosphamide).[14]

Newer therapies may be effective even with fewer doses and therefore they could reduce patient burden and length of treatment. Anti-TROP2 ADCs have shown significant benefit in the metastatic setting for patients with TNBC, so it is reasonable to expect similar benefit in the neoadjuvant/WOO setting in the future, perhaps with a shorter treatment duration. The NeoSTAR phase II trial published the first results recently, in which the neoadjuvant sacituzumab govitecan single-arm cohort showed a 30% pCR (with TILs and Ki67 as predictors but not TROP2), ORR 64%, and 2-year EFS of 95% (100% for patients with pCR).[62] The I-SPY arm datopotamab deruxtecan (Dato-Dxd) + durvalumab presented at ASCO 2024 has shown that after four cycles, 33% of patients were able to skip traditional chemotherapy and proceed straight to surgery, and that 72% patients with the HER2-immune+ subtype achieved pCR.[63] There are other interesting chemotherapy drugs entering clinical development, including INT230-6, a novel formulation of cisplatin, vinblastine, and a tissue dispersion enhancer designed for intratumoral delivery, which seems to cause significant intratumoral necrosis and stimulates immune response.[64]

HOW TO TREAT COLD TUMORS

Immunologically speaking, tumors can be divided into three phenotypes: (i) cold, characterized by the complete absence of immune cells; (ii) excluded, in which the presence of the immune cells is restricted to the periphery but absent within the tumor microenvironment; and (iii)

inflamed, where the immune cells are present in the periphery, stromal compartment and/or in the intratumoral area. Patients with TNBC who have the worst prognosis are those with cold tumors, and exhibit relapse rates of ~30%.[65]

Cold tumors are challenging to treat because of the difficulty in provoking antigen presentation due to barriers like stiff stroma, low mutational burden or immunosuppressive microenvironment that has impeded immune infiltration in the first place.[66] Therefore, patients with tumors that have low TILs might need a different strategy compared with those with high TILs. Novel immunotherapy approaches have the potential to induce immune recognition and infiltration in these cold tumors, but may require the use of a combination of immunotherapies or the addition of other therapies such as vaccines, cytokines, PARP inhibitors, cryoablation, radiotherapy or stereotactic body radiation therapy (SBRT), anti-vascular endothelial growth factor (lenvatinib) or anti-DNA damage response (DDR) agents.

Antigen presentation can be enhanced by promoting tumor cell apoptosis or by causing new mutations in the tumor DNA. The combinations of ipilimumab + cryotherapy or pembro + radiotherapy have the potential to cause the release of neoantigens that can activate naive T cells.[21,22] There are ongoing WOO trials assessing nivolumab + ipilimumab + cryotherapy (NCT03546686), pembro + intraoperative radiation therapy (NCT02977468) or a new toll-like receptor 9 (TLR9) agonist immunotherapy called CMP-001 combined with SBRT (NCT04807192). PARP inhibitors can increase the number of mutations in tumor cells, which has been proved to be effective in treating g*BRCA*m or HR-deficient tumors in the WOO trial combining olaparib with durvalumab.[41] New drugs like INT230-6 result in increased TILs and other immune markers even in immune quiescent tumors like estrogen receptor (ER)+ breast cancers.[67]

The TONIC trial has assessed different strategies to induce immune activation in patients with metastatic TNBC: (i) without induction, (ii) with 2-week low-dose chemotherapy induction, (iii) with irradiation, (iv) cyclophosphamide, (v) cisplatin or (vi) doxorubicin, all followed by nivolumab. The highest response rate was observed with cisplatin (35%) and doxorubicin (20%), in which the investigators observed an increase of PD-(L)1 and T cytotoxic cell pathways.[68] Although this was tested in the metastatic setting, a similar strategy could be also applied in patients with early TNBC with low TIL count.

In some patients, T cells may recognize cancer cells, but get switched off at the regional lymph nodes and are thus unable to migrate to the tumor site. Anti-cytotoxic T lymphocyte associated antigen-4 (anti-CTLA-4) drugs are known to act on the lymph nodes and activate these T cells leading to immune recognition. It has been shown that patients with TNBC who have low TILs (≤5%) have a statistically higher CTLA-4 expression in the T cells of the lymph nodes and more neoantigens in the tumor than patients with higher TILs (≥50%).[69] This highlights the importance of potentially reactivating immune cells and releasing them from the regional lymph nodes with anti-CTLA-4 therapy, which can be combined or given sequentially with other ICIs, new immunotherapies like the multicytokines IRX-2, interleukin 2 (IL-2) or CMP-001 (TLR9 agonist) drugs, or virotherapies currently being tested in breast cancer.[70] The I-SPY platform trial (NCT01042379) that tests combinations of experimental drugs with standard chemotherapy, is evaluating a new virotherapy VSV-IFN-β-NIS plus the anti-PD-1 agent cemiplimab, and results are eagerly awaited.

WHEN AND WHAT ADJUVANT TREATMENT IS NEEDED?

The comparison between neoadjuvant and adjuvant immunotherapy in patients with melanoma demonstrated that immunotherapy treatments seem to be more effective in the presence of the tumor.[71] This could be explained by the release of neoantigens due to the apoptosis of cancer cells that can trigger new antigen presentation and the formation of new T-cell clones. Another hypothesis is that one or several regional lymph nodes, which may potentially contain lymphocytes that have recognized the tumor, are removed during surgery or irradiated with radiotherapy, making the immunotherapy less effective as they cannot reactivate many lymphocytes that have previously encountered a tumor neoantigen. Also, adjuvant immunotherapy can cause more off-target immune activation as there is no tumor, and therefore higher number of irAEs. This can put at risk the patient's quality of life and, in a small proportion of cases, could even result in death. The Impassion-030 study, where they evaluated the addition to atezolizumab (anti-PD-L1) to adjuvant paclitaxel and anthracyclines followed by atezolizumab monotherapy in maintenance in early TNBC patients, did not show any improvement on EFS in either the intended to treat population nor in the PD-L1- or node-positive subgroups.[72]

The standard of care for early TNBC after NACT + pembro followed by surgery is to complete 1 year of adjuvant pembro in all patients regardless of the pathological response. Other drugs approved in the adjuvant setting for TNBC include capecitabine for patients with residual disease after NACT,[4] and olaparib for patients with g*BRCA*m.[3] Currently, there is a debate regarding the best treatment of patients with non-pCR and pCR.

For patients who achieve pCR, there is an opportunity to deescalate adjuvant treatment with immunotherapy. The OptimICE-pCR trial (NCT05812807) is currently comparing pembro versus observation to test this hypothesis.

For patients who do not achieve pCR, we need better strategies given the poor outcomes for these patients. On the one hand, several trials are questioning if there is a need for further immunotherapy at all for non-pCR patients. The A-BRAVE trial compares avelumab versus observation,[73] the SWOG S1418 study is assessing pembro versus observation,[74] and the cTRAK trial is randomizing patients with residual disease and ctDNA+ to pembro versus observation.[75] The A-BRAVE trial recently reported results in which the 3-year EFS was 68.3% versus 63.2% ($P = 0.172$), and the

3-year OS was 84.8% versus 76.3% ($P = 0.035$).[76] On the other hand, trials are comparing different combinations of immunotherapy, capecitabine, olaparib, and other drugs for patients with non-pCR and/or ctDNA-positive status at surgery. Some examples are TROPION-Breast03 (NCT05629585), PERSEVERE (NCT04849364), ARTEMIS (NCT04803539), Apollo (NCT04501523), ASPRIA (NCT04434040), ASCENT-05/OptimICE-RD (NCT05633654), SASCIA (NCT04595565), OXEL (NCT03487666), and ZEST (NCT04915755). Especially interesting is the TROPION-Breast03 trial, which is comparing adjuvant Dato-Dxd, Dato-Dxd + durvalumab versus investigator's choice of therapy (capecitabine, pembro or capecitabine + pembro).

CONCLUSIONS AND FUTURE PERSPECTIVES

In this article, we have summarized and reviewed the results of key WOO trials assessing ICIs in early-stage TNBC disease. As discussed, some patients have received a benefit of immunotherapy alone or combined with other non-chemotherapy treatments in several trials. This opens the possibility of de-escalating NACT and immunotherapy partially or completely in a selected population, and therefore decreasing the occurrence of adverse events from both therapies. Biomarkers like TILs, PD-L1, and IFN-γ signature could help to identify which patients are likely to benefit from one or few doses of immunotherapy alone, a combination with other immunotherapies or targeted therapies.

pCR has historically been a surrogate biomarker for EFS and OS in NACT trials in TNBC, but current evidence suggests that it may not be the best marker to assess the benefit of immunotherapy in this setting. Biomarker changes in the tumor, like the increase of TILs, increase of PD-L1 expression or TCR expansion are emerging as potential biomarkers to assess the long-term response of the immunotherapy, although this would require performing a post-treatment biopsy. TCR expansion and ctDNA clearance in the blood could also effectively monitor the response to neoadjuvant strategies with an easier access than the tumor tissue.

Certain patients with low TILs (cold tumors) could receive drugs that can help the induction of lymphocytes into the tumor microenvironment. A combination of immunotherapies with radiotherapy (intraoperative radiotherapy, SBRT), cryotherapy, targeted agents like PARP inhibitors (for g*BRCA*m or patients with HR-deficient tumors) or anti-DDR drugs could increase the number and the activity of TILs. New immunotherapies targeting other immune checkpoints like TIGIT, TIM3, LAG3, OX-40 or B7.H4, IL-2, multi-cytokines IRX-2, or CMP-001 are currently being assessed in trials and could provide benefit for both high and low TIL tumors. There is a need to identify novel effective biomarkers that can help guide therapy with these novel emerging immunotherapies; genomic signatures might also play a role in the predictive algorithms to assess activity with different immunotherapy-based strategies.

The dream of chemotherapy de-escalation with immunotherapy-based approaches seems to be closer, at least in a minority of carefully selected patients. TNBC is very sensitive to taxanes and platinum compounds, so it would be clinically appropriate to explore reducing or eliminating anthracyclines when a patient has had an increase of TILs, PD-L1, major response and/or ctDNA clearance after neoadjuvant taxanes, carboplatin, and pembro. New promising drugs like anti-TROP ADCs will hopefully be more effective with potentially shorter duration and improved toxicity profiles.

The need for adjuvant treatment with ICIs is controversial, especially after the approval of both adjuvant and neoadjuvant pembro together, regardless of the pathological response. This regimen has raised debates in the oncology community, with ongoing trials assessing the need for adjuvant immunotherapy, especially for patients with pCR. Given that capecitabine is approved for TNBC patients with non-pCR and olaparib for g*BRCA*m patients independent of the pathological response, some of these trials compare further immunotherapy with these treatments in the specific patient populations.

Reducing NACT treatment could also reduce the number of immunotherapy doses in comparison to the current standard of care, which has several benefits. The possibility of only needing a few doses before surgery, or less immunotherapy when de-escalating NACT, offers the advantage of limiting the possibility of irAEs. It also makes the treatment more affordable and therefore increases patients' access to immunotherapy. Shortening the duration of the therapy will also improve patients' quality of life and will reduce the number of hospital visits and procedures.

In conclusion, fewer doses of immunotherapy alone before chemotherapy may be beneficial in certain patients with early-stage TNBC. At this point, many of these WOO studies are small and hypothesis generating, so larger prospective trials with this approach are needed with ongoing biomarker evaluation. Tailoring further treatment based on changes in the tumor environment and response to immunotherapy should be investigated. These approaches may facilitate a partial de-escalation of cytotoxic chemotherapy and immunotherapy in the neoadjuvant setting in some patients without compromising their long-term prognosis.

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