

Article

Not peer-reviewed version

Neoadjuvant Pembrolizumab Associated with Chemotherapy in Early Breast Cancer Patients: Real-World Data from a French Single-Center Experience

[Ichrak Ben Abdallah](#)^{*}, [Severine Guiu](#), [Xavier Quantin](#), [William Jacot](#), Philine Witowski

Posted Date: 8 December 2025

doi: 10.20944/preprints202512.0731.v1

Keywords: pembrolizumab; triple negative breast cancer; toxicity



Preprints.org is a free multidisciplinary platform providing preprint service that is dedicated to making early versions of research outputs permanently available and citable. Preprints posted at Preprints.org appear in Web of Science, Crossref, Google Scholar, Scilit, Europe PMC.

Copyright: This open access article is published under a [Creative Commons CC BY 4.0 license](#), which permit the free download, distribution, and reuse, provided that the author and preprint are cited in any reuse.

Disclaimer/Publisher's Note: The statements, opinions, and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions, or products referred to in the content.

Article

Neoadjuvant Pembrolizumab Associated with Chemotherapy in Early Breast Cancer Patients: Real-World Data from a French Single-Center Experience

Ichrak Ben Abdallah ^{1,*}, Severine Guiu ^{1,2}, Xavier Quantin ^{1,2}, William Jacot ^{1,2} and Philine Witowski ¹

¹ Institut de Recherche en Cancérologie de Montpellier, Montpellier, France

² IRCM, Univ Montpellier, ICM, INSERM, Montpellier, France

* Correspondence: ichrak.ben-abdallah@icm.unicancer.fr

Simple Summary

This retrospective study assessed real-world feasibility, safety, and efficacy of neoadjuvant pembrolizumab plus chemotherapy for early triple-negative breast cancer (TNBC) at Montpellier Cancer Institute (2022–2024). Ninety-two patients were included (median age 50). Grade 3–4 immune-related adverse events (irAEs) occurred in 20%, most commonly hepatitis, colitis, skin toxicity, myocarditis, and rare hematologic or endocrine events; no treatment-related deaths were reported. One third of patients discontinued immunotherapy because of irAEs. The pathological complete response (pCR) rate was 61.1%, with no association between pCR and number of pembrolizumab cycles. Patients experiencing grade 3–4 irAEs showed a trend toward higher pCR (80% vs 56.7%). Baseline antinuclear antibodies were not linked to increased toxicity. Overall, real-world toxicity and efficacy were consistent with KEYNOTE-522 findings.

Abstract

Introduction: The addition of immunotherapy to neoadjuvant treatment for early triple-negative breast cancer (TNBC) has been adopted in clinical practice in France since March 2022 with little real-world data published on the topic. The aim of this study was to evaluate real world data on treatment feasibility, efficacy and related toxicities, with a specific focus on immune-related adverse events (irAEs). **Methods:** We conducted a retrospective analysis of patients who completed at least the neoadjuvant sequence of pembrolizumab combined with chemotherapy for early-stage TNBC at Montpellier Cancer Institute from April 2022 to July 2024. Adverse events were graded according to the Common Terminology Criteria for Adverse Events (CTCAE) v5.0. The pathological complete response (pCR) was defined as the absence of residual invasive disease in the breast and axillary lymph nodes (ypT0/Tis ypN0). **Results:** We reviewed data from 92 patient records. The median age at diagnosis was 50 years (range: 27–76). The history of autoimmune disease was noted in 3.2%. Grade 3–4 irAEs were observed in 20% of patients and included hepatitis (8.6%), colitis (3.3%), skin toxicity (2.1%), myocarditis (2%), arthralgia (1%), autoimmune hemolytic anemia (1%), hypothyroidism (1%), and adrenal insufficiency (1%). No treatment-related deaths were reported. Immunotherapy was discontinued due to irAEs in 1/3 of patients of the study population. pCR rate was 61.1%, with no significant association between the number of neoadjuvant pembrolizumab cycles and pCR rate ($p=0.7$). Patients experiencing grade 3–4 irAEs had a pCR rate of 80%, compared to 56.7% in those without such toxicities ($p=0.079$). Initial positivity of antinuclear antibodies (ANA) was not associated with an increased incidence of irAEs. **Conclusion:** The immune-related adverse events and efficacy data observed in our cohort were broadly comparable to those reported in the KEYNOTE-522 trial with no treatment related deaths. Patients with grade 3–4 irAEs tended to have higher pCR rates.

Keywords: pembrolizumab; triple negative breast cancer; toxicity

1. Introduction

Breast cancer is diagnosed at a localized stage in approximately 60% of cases in France [1]. Triple-negative breast cancers (TNBC) account for around 15% of all breast cancers [2]. Immunotherapy has found its first clinical indication in this subpopulation due to specific immunogenic features: high levels of tumor-infiltrating lymphocytes (TILs), a high mutational burden, and increased PD-L1 expression compared to other subtypes [3].

Immune checkpoint inhibitors (ICIs) were initially validated in metastatic or unresectable locally advanced TNBC. In 2021, pembrolizumab received marketing authorization for metastatic first-line use in combination with chemotherapy based on the results of the KEYNOTE-355 trial [4].

In the neoadjuvant setting, the KEYNOTE-522 study [5] changed the standard of care by demonstrating a benefit from the addition of pembrolizumab (anti-PD-1) to chemotherapy. In France, access to this regimen was initially granted through an early access authorization in March 2022 [6], with official reimbursement following in November 2024 [7].

Given this treatment escalation integrating immunotherapy to chemotherapy in a neoadjuvant setting, the main objective of this study was to assess the tolerance of this regimen, with a focus on immune-related toxicities, their incidence, management and impact on treatment course.

2. Materials and Methods

2.1. Study Design and Setting

This is a retrospective descriptive study involving patients treated at the Montpellier Cancer Institute for localized TNBC according to the Keynote 522 regimen [8]. Eligibility required having received neoadjuvant pembrolizumab-based therapy, followed by surgery (except in cases where surgery was not performed due to disease progression).

2.2. Data Collection and Analysis

Collected data included demographic characteristics, clinical and biological features of the disease, and treatment details. Toxicities were graded according to the Common Terminology Criteria for Adverse Events (CTCAE) V 5.0. [9]. Statistical analyses were conducted using SPSS software version 26. Descriptive statistics included means and quartiles for continuous variables, and frequencies for categorical variables.

3. Results

3.1. Patient Characteristics and Pretreatment Assessment

A total of 92 patients were included in the study. Epidemiological characteristics are presented in Table 1. The median age at diagnosis was 50 years (range: 27–76), with 24% under 40 years and 3.2% over 70 years. At baseline, three patients had pre-existing autoimmune disorders: one with rheumatoid arthritis, one with eczema, and one with Hashimoto’s thyroiditis. Pre-treatment biological data, including serum autoantibodies, are summarized in Table 2.

Table 1. Epidemiological characteristics of the study population.

Parameter	n (%) / Values
Age (years)	
Mean [range]	50 [27–76]
Median [range]	50 [27–76]
Performance status (PS)	

PS0	96.7
PS1	3.3
Menopausal status	
Menopausal	47.8
Premenopausal	46.8
Perimenopausal	5.4
History of hormonal intake	
Yes	13.0
No	29.0
Unknown	58.0
Active smoking	
Yes	34.4
No	44.1
Unknown	21.5
Alcohol intake	
Yes	9.7
No	44.1
Unknown	46.2
BMI	
Mean [range]	25.2 [17–41.79]
Median	24.17
Comorbidities*	
Yes	18.3
No	77.4
History of autoimmune disease	
Yes	3.3
No	96.7
Personal history of breast cancer	
Yes	7.6
No	92.4
Pathogenic BRCA1/2 mutations	
BRCA1	9.8
BRCA2	1.1

BMI: body mass index. ***Comorbidities** include hypertension, diabetes, dyslipidemia, renal insufficiency, history of ischemic disease (heart disease, stroke, peripheral arterial disease), and thyroid dysfunction.

Table 2. Biological data and anti-immune antibodies at baseline.

Parameter	n (%)
LDH	
Elevated	7.7
Normal	54.3
Unknown	38
Albumin	

Normal	65
Unknown	35
Antinuclear antibodies (ANA)	
Positive	17.4
Negative	39.1
Unknown	43.5
Rheumatoid factor	
Positive	3.3
Negative	47.8
Unknown	48.9
Anti-CCP Ab	
Positive	0.0
Negative	45.7
Unknown	54.3
Anti-TPO Ab	
Positive	5.4
Negative	45.7
Unknown	48.9

LDH: lactate dehydrogenase; **anti-CCP Ab:** anti-cyclic citrullinated peptide antibodies; **anti-TPO Ab:** anti-thyroid peroxidase antibodies.

3.3. Overall Treatment Tolerance

In the neoadjuvant phase, 68% of patients completed all 8 planned neoadjuvant pembrolizumab cycles. Only 43% of patients were able to complete all 12 cycles of paclitaxel–carboplatin. The median number of neoadjuvant paclitaxel–carboplatin cycles received was 11. Treatment interruptions or delays due to clinical and/or laboratory toxicity during this regimen were observed in 67% of patients. During the anthracyclines sequence, 79.3% of patients completed all four planned cycles.

In the adjuvant phase, patients who received pembrolizumab accounted for 65% of the cohort. Table 4 presents the various reasons for not administering adjuvant immunotherapy in the remaining patients (N=32).

Table 3. Reasons for non-administration of pembrolizumab in the adjuvant setting (N= 32).

Reason for non-administration of treatment	100% (N=32)
Immunotherapy-related toxicity occurring during the neoadjuvant phase (irrespective of severity)	50%
Isolated troponin elevation on the pre-treatment workup for adjuvant therapy, with normal findings on cardiological investigations	18,8%
Disease progression	6,2%
Pathological complete response and decision not to pursue with the adjuvant sequence	9,4%
Patient’s preference	12,5%
Anthracycline-related cardiac toxicity, with a preference to avoid adding immunotherapy in this context	3,1%

The main toxicities observed during treatment are presented in Table 5. It is noteworthy that immunotherapy was discontinued due to potentially immune-related toxicity in 29.3% of cases. No deaths related to immunotherapy or chemotherapy were reported during the treatment period. Grade 3–4 irAEs affected 20% of the study population and are the followings: hepatitis (8,6%), colitis (3,3%), skin toxicities (2,1%), myocarditis (2%), arthralgias (1%), autoimmune hemolytic anemia (1%), hypothyroidism (1%), adrenal insufficiency (1%).

No association was found between the occurrence of grade 3–4 toxicities and baseline antinuclear antibody status (positive or negative).

Table 5. Frequency of main toxicities observed according to treatment sequences including pembrolizumab (neoadjuvant and adjuvant phases).

Toxicity	PC-pembrolizumab (N=92)		EC-pembrolizumab (N=91)		Adjuvant pembrolizumab (N=60) ^a	
	Grades 1-2	Grades 3-4	Grades 1-2	Grades 3-4	Grades 1-2	Grades 3-4
Allergic reaction	18,5%	0	4,3%	0%	0%	0%
Skin toxicity*	23,9%	1,1%	9,8%	0	5%	1,7%
Diarrhea	38%	2,2%	8,7%	1,1%	3,3%	0%
Nausea	58,7	2,2%	52,2%	1,1%	0%	0%
Vomiting	16,3%	1,1%	17,4%	0%	0%	0%
Peripheral neuropathy	41,3%	1,1%	0%	0%	0% **	0%
Arthralgias	9,8%	0%	5,4%	0%	6%	1,6%
Myalgias	18,5%	0%	1,1%	0%	0%	0%
Mucitis	13%	0%	15,2%	1,1%	0%	0%
Constipation (de novo)	20,7%	0%	7,6%	0%	0%	0%
Thrombopenia	12%	1,1%	4,3%	1,1%	0%	0%
Anemia	58,7%	4,3%	53,3%	6,5%	0%	0%
Neutropenia	20,7%	43,5%	12%	18,5%	0%	0%
Febrile neutropenia	2,2%		17,4%		0%	0%
Fever	16,3%	12%	19,6	1,1%	0%	0%
Infections	22,8%	0%	14,1%	0%	5%	0%
Elevated ALT levels	16,5%	5%	4%	3%	15%	0%
Elevated Troponin levels	1%	0%	6,5%	0%	***	1,6%
Dysthyroidism (de novo)	12%	0%	7,6%	1%	8,3%	0%
Adrenal insufficiency	3,2%	0%	2,1%	1%	3,3	0%
Renal toxicity	1%	0%	0%	0%	0%	0%
AIHA	0%	1%	0%	0%	0%	0%
Sicca syndrome	1%	0%	2,1%	0	1%	0%
Carpal tunnel syndrome	0%	0%	0%	0%	1%	0%

PC: Paclitaxel-Carboplatine. **EC:** Epirubicine-Cyclophosphamide. **ALT:** alanine aminotransferase. **AIHA:** autoimmune hemolytic anemia. **a:** Percentages are calculated based on the number of patients who received adjuvant pembrolizumab, N = 60. * Cutaneous toxicity does not include nail or hair toxicity. ** Pre-existing residual neuropathies were not taken into account; only de novo toxicities were recorded. *** Patients with troponin abnormalities during the pre-treatment workup for the adjuvant phase were not included; this issue will be addressed in the section dedicated to cardiac toxicity.

3.4. Liver Toxicity

Thirty-six percent (36%) of the patients in the study experienced liver function test abnormalities during their treatment, with 31.5% occurring during the neoadjuvant phase. These were grade 1–2 in 29.3% of cases, and grade 3–4 in 8.6%, including 2.2% of grade 4, according to the CTCAE v5.0 classification. A liver biopsy was deemed necessary in 9.4% of the cases, confirming immune-related hepatitis. The biological characteristics, severity grading, management, and clinical outcomes are detailed in Table 6.

It is worth noting that no significant association was found between the presence of antinuclear antibodies (ANA) at baseline and the occurrence of liver function test abnormalities (p = 0.52). Similarly, there was no association between the grade of hepatic involvement and ANA status

(positive or negative) at the time of toxicity (p = 0.6). Finally, all patients who presented with positive ANA at the time of hepatic toxicity had negative ANA at baseline.

Table 6. Characteristics of Liver Toxicity Related to Treatment.

N=33(=100%)	Grade 1 (58%, N=19)	Grade 2 (18% ,N=6)	Grade 3 (18% , N=6)	Grade 4 (6%, N=2)
Time to onset *	15,8 [1-57]	5,5 [1-9]	6 [1-18]	20,5 [1-40]
Presentation				
Cholestatic	42,4%	0%	3%	0%
Cytolytic	3%	0%	12%	0%
Mixed	12%	18%	3%	6%
Immune antibodies				
Negative	15%	12%	15%	6%
Positive	ANA* 9%	0%	ANA* 3%	0%
Not tested	34%	6%	0%	0%
Infectious serology tests				
Positive	0%	0%	0%	0%
Negative	27%	18%	18%	6.1%
Not tested	31%	0%	0%	0%
Biopsy	No	No	3%	6,1%
Corticosteroids	0%	Yes 3% No 15%	Yes 3% No 15%	Yes 6,1%
Delursan	0%	0%	0%	6,1%
Clinico-biological course.	Favorable outcome	Favorable outcome	Favorable outcome	Favorable outcome

* in weeks; *ANA (Anti-nuclear Antibodies) detected at a titer of 1:160 with a speckled pattern, but serum was negative for autoimmune hepatitis–specific antibodies.

3.5. Cutaneous Toxicity

Cutaneous toxicity (excluding nail disorders and other adnexal abnormalities attributable to chemotherapy) was observed in 32.6% of patients during the study. Table 7 summarizes the various cutaneous manifestations observed.

Table 7. Characteristics of Observed Cutaneous Toxicities (N = 30; 100%).

Variable	Value
Time to onset (weeks)	
Median	2.5 [1–88]
Mean	11.8
Type of skin lesion	
Isolated pruritus	26.7% (8%*)
Maculopapular exanthem (rash)	23.3% (7.7%*)
Isolated skin xerosis	6.7% (2%*)
Urticaria	16.7% (5.4%*)
Eczema	3.3% (1%*)
Acneiform eruption	3.3% (1%*)
Psoriasis	3.3% (1%*)
Disabling lichen	10% (3.2%*)
Eczema + Acneiform eruption	3.3% (1%*)

Suspicion of DRESS syndrome	3.3% (1%*)
Clinical Grade (CTCAE v5.0)	
Grades 1–2	93.3% (30.4%*)
Grades 3–4	6.7% (2.2%*)

*Percentage of the event relative to the total study population of 92 patients. **CTCAE v5.0:** Common Terminology Criteria for Adverse Events, version 5.0.

3.6. Endocrine Toxicity

New-onset thyroid dysfunction occurred in 27% of patients, including 14% with initial hyperthyroidism and 25% with hypothyroidism.

New-onset adrenal insufficiency was reported in 8.6% of cases. These toxicities are primarily attributed to immunotherapy due to their underlying mechanisms. Table 8 summarizes data regarding the timing of onset, severity grade, and associated immunological features.

Table 8. Characteristics of Immune-Related Endocrine Toxicities (Thyroid Dysfunction and Adrenal Insufficiency).

	Thyroid dysfunction (N = 25; 100%)	Adrenal insufficiency (N = 8; 100%)
Time to onset (weeks)		
Mean	17 [4–44]	17.6 [8–33]
Median	14	13
Timing of onset		
Neoadjuvant	80% (21.7% of totalcohort)	75% (6/8)
Adjuvant	20% (5.4% of total cohort)	25% (2/8)
Severity (CTCAE v5.0)		
Grades 1–2	95.7% (26% of total cohort)	87.5% (7/8)
Grades 3–4	4.3% (1 % of total cohort)	12.5% (1/8)
Anti-TPO antibodies		
Positive	16%	
Negative	32%	
Not tested	52%	
Toxicity requiring hospitalization	4%	12.5% (1/8)
Anti-TPO Ab: Anti-thyroid peroxidase antibodies; CTCAE v5.0: Common Terminology Criteria for Adverse Events, version 5.0.		

3.7. Colitis

Diarrhea was reported in 44.6% of patients, occurring predominantly (95.2%) during the neoadjuvant phase. It was grade 1–2 in 92.7% of cases. All patients who experienced grade 3–4 diarrhea (N = 3, i.e., 3% of the cohort) underwent gastrointestinal endoscopy with biopsies.

To provide a clearer understanding of these severe events, the clinical, endoscopic, and histological characteristics of the three affected patients are detailed below.

Patient A (58 years old) presented with grade 1–2 diarrhea starting at week 3 and progressing to grade 3 by week 10. Endoscopy revealed erythematous mucosa with bleeding on contact but no ulceration. Biopsy showed inflammatory colitis consistent with an immune-related mechanism. Cytomegalovirus (CMV). Immunohistochemistry (IHC) was negative, whereas CMV PCR (polymerase chain reaction) on biopsy was positive, suggesting viral superinfection. The patient was treated with valganciclovir, followed by oral corticosteroids, and ultimately a TNF (Tumor Necrosis Factor) inhibitor due to corticosteroid dependence.

Patient B (44 years old) developed diarrhea at week 4, worsening to grade 3 by week 20. Endoscopy showed severe proctitis and colitis with superficial ulcerations. Biopsy revealed diffuse

acute colitis of undetermined origin, with negative CMV IHC but a weakly positive CMV PCR on rectal biopsy. Serology was negative (IgM and IgG). Clinical improvement occurred after a 15-day course of valganciclovir followed by oral corticosteroids.

Patient C (46 years old) presented at week 3 with grade 3 diarrhea associated with rectal bleeding, followed by constipation. Endoscopic evaluation of the rectum and colon was normal, aside from the presence of hemorrhoids and an anal fissure. Histological analysis showed moderate chronic interstitial follicular proctitis, judged unlikely to be immune-related. The symptoms resolved with symptomatic treatment alone.

3.8. Cardiac Toxicity

An elevation of the troponin level was observed in 16.3% of patients (N = 15). A formal diagnosis of myocarditis was confirmed in only two patients (2%), referred to as Patient A and Patient B later in the text.

Notably, elevated troponin levels were detected in 9 patients (9.7% of the total cohort) during the pre-treatment assessment for the adjuvant phase, within a postoperative context. Among them, one (Patient A) underwent a cardiac MRI that revealed features consistent with myopericarditis highly suggestive of an immune-related etiology, according to the Lake Louise criteria [10]. The eight other patients (8.6%) were not administered pembrolizumab in the adjuvant setting, following the precautionary recommendation of their referring oncologist. Their troponin levels returned to normal range during follow-up.

As for Patient B, she exhibited a troponin elevation to 35 pg/mL (compared to <10 pg/mL at baseline) during adjuvant pembrolizumab, despite normal findings on echocardiography, cardiac MRI, and electrocardiogram (ECG), an endomyocardial biopsy was performed revealing nonspecific myocarditis with a minimal lymphocytic infiltrate. These results reflected either a pauci-lesional form of immune-mediated myocarditis or a benign postoperative inflammatory reaction. Considering that surgery and myocardial biopsy were separated by a 5-month interval, postoperative context-related myocarditis is unlikely. The immunotoxicity board therefore established the diagnosis of immune-mediated myocarditis.

3.9. Pathological Response

A pathological complete response (pCR) was achieved in 61.1% of patients. The distribution of pathological responses according to the Residual Cancer Burden (RCB) score was as follows: RCB score 0 (57.6%), score 1 (7.6%), score 2 (16.3%), score 3 (3.3%), and not assessable (15.2%).

Patients who experienced grade 3–4 immune-related toxicities tended to have a higher pCR rate compared with those without such toxicities (80% vs. 56.8%). A one-sided Fisher's exact test indicated a trend toward this association ($p = 0.079$), although the difference did not reach conventional statistical significance.

There was no significant association between pCR rate and the number of neoadjuvant pembrolizumab cycles received ($p = 0.7$).

Survival analyses (disease-free survival and overall survival) and the impact of grade 3–4 toxicities on survival remain immature (median follow-up of 22 months) and will be addressed separately after a longer follow-up.

4. Discussion and Conclusions

In this study, we evaluated the real-world tolerability of the new neoadjuvant immunochemotherapy regimen (KEYNOTE-522 protocol) in patients with localized TNBC.

In our cohort, the initial phase of the protocol combining pembrolizumab, carboplatin, and paclitaxel (PCP) was marked by numerous treatment delays and cancellations, mainly due to hematological toxicities. Grade 3–4 neutropenia was the most frequent adverse event, occurring in 43.5% of patients, although febrile neutropenia remained rare (2.2%). Conversely, the epirubicin–

cyclophosphamide–pembrolizumab (ECP) sequence was associated with a higher incidence of febrile neutropenia (17.4%).

Beyond hematologic events, the most common adverse events during the PCP phase included hypersensitivity reactions, skin rash, peripheral neuropathy, diarrhea, and elevated alanine aminotransferase (ALT) levels.

The most frequent grade 3–4 immune-related adverse events (irAEs) in our cohort were, in decreasing order: hepatitis (8.6%), colitis (3.3%), dermatologic toxicity (2.1%), myocarditis (2%), disabling arthralgia (1%), autoimmune hemolytic anemia (1%), life-threatening hypothyroidism (1%), and adrenal insufficiency (1%). No cases of immune-related pneumonitis were reported. Pembrolizumab was discontinued due to toxicity in 29.3% of patients in our study—a higher rate than that reported in KEYNOTE-522, where 20% discontinued due to adverse events, most commonly pneumonitis (1.9%), diarrhea (1.7%), and elevated transaminases (1%) [11]. However, this discontinuation rate aligns with other real-world data, coming from the Gustave Roussy, which reported a 35% discontinuation rate in 63 patients [12]. This discrepancy between real-world and clinical trial data may reflect a more preventive approach adopted by oncologists in curative settings for young patients, where the margin for error is minimal—particularly in the neoadjuvant setting, where a severe or prolonged complication could jeopardize subsequent treatment steps, including surgery. In addition, patients treated in routine care are less selected than those enrolled in prospective trials, often presenting with comorbidities or baseline fragilities that increase the likelihood of treatment interruption.

A notable finding in our series was the detection of elevated troponin levels in 9 patients (9.7% of the cohort) after surgery, during the pre-adjuvant phase treatment assessment. In one of them, cardiac MRI suggested immune-mediated myocardial involvement, which improved without corticosteroid (declined by the patient). In the remaining 8 patients (8.6%), adjuvant pembrolizumab was withheld as a precaution based on their oncologists' recommendation. Of note, troponin levels returned to normal range during follow-up. This frequent troponin elevation in the postoperative setting may be consistent with the phenomenon known as myocardial injury after non-cardiac surgery (MINS), which occurs in 10–25% of cases [13]. Other possible explanations include anthracycline-induced cardiotoxicity administered before surgery, or non-ischemic myocardial injury related to systemic inflammation caused by the disease itself.

Among the 92 patients in our cohort, only two had a diagnosis of myocarditis (one probable immune-mediated in the postoperative setting as described above, and another with a pauci-lesional myocardial biopsy suggestive of immune-mediated injury during adjuvant therapy), representing 2.2% of our cohort. Both cases were incidentally detected through mild troponin elevation and had favorable outcomes. This rate is consistent with, or slightly higher than, published reports. Indeed, in the KEYNOTE-522 trial, immune-mediated myocarditis occurred in less than 1% of patients (0.4%; 3 cases among 784 patients) [14]. These observations underscore the importance of biological monitoring, even in asymptomatic patients, although the benefit of systematic myocarditis screening during immunotherapy remains debated [15].

Grades 3–4 hepatic toxicity in our cohort accounting for 8.6% was broadly comparable to that reported in the KEYNOTE-522 trial (5.2%) [16] and to real-world data [12]. Regarding biochemical phenotype of hepatitis, cholestatic forms were most common across all grades (42.4%), followed by mixed forms (39%) and cytolytic forms (15%). However, in severe cases (grades 3–4), cytolytic patterns predominated. This distribution aligns with literature findings, particularly the multicenter study by Lina Hountondji *et al.* [17], which reported that PD-1 inhibitor-related hepatitis most frequently presents with a cholestatic or mixed pattern, whereas cytolytic forms are more often associated with anti-CTLA-4 combinations. No patients had positive serum antibodies for autoimmune hepatitis at the time of toxicity. However, antinuclear antibodies (ANA) were detected in 12% of patients at the time of hepatic injury (9% in grade 1 cases, 3% in grade 3 cases), whereas all these patients were ANA-negative at baseline. This phenomenon, suggesting secondary induction of autoimmunity, has been described in the literature. Nilasha *et al.*, reported autoantibody positivity

in approximately 18% of patients with ICI-related hepatitis, with no proven prognostic or predictive value [18]. It should be noted that our grading relied on CTCAE v5.0 criteria, as routinely used for immune-related hepatitis. These criteria do not always reflect the true clinical impact of toxicity, as suggested by L. Meunier *et al.*, who recently advocated for alternative hepatology-derived scores (MELD, DILI-IEWG) to better guide therapeutic decisions and assess whether immunotherapy can be safely resumed [19]. We emphasize the critical role of pathological assessment in cases of grade 3–4 liver enzyme elevations during the neoadjuvant phase, given the challenge of distinguishing chemotherapy-induced hepatotoxicity from pembrolizumab-related immune-mediated toxicity.

Regarding cutaneous toxicity, our findings are consistent with KEYNOTE-522 [20], with most cases being maculopapular rash and isolated pruritus, typical of anti-PD-1 therapy [21].

Thyroid dysfunction was observed in 27% of patients—higher than in KEYNOTE-522 (8% hypothyroidism, 3.4% hyperthyroidism) [20]—but in line with other real-world reports (France: 29%, United Kingdom: 26.7%) [12,22]. We found no association between baseline anti-TPO antibody positivity and the development of thyroid dysfunction, contrary to some literature reports [23], possibly due to missing data (half of the patients lacked baseline anti-TPO measurement), which limits interpretation.

Regarding gastrointestinal toxicity, diarrhea was frequent (44.6% vs. 30.4% in KEYNOTE-522) [19], but severe colitis (grade 3) was rare (3% vs. 2.6%). No grade 4 colitis was reported. Among the three patients with grade 3 colitis, two had cytomegalovirus (CMV) positivity on biopsy, complicating attribution solely to immunotherapy. Literature describes three possible mechanisms for the CMV–colitis association in this setting [23]: pure CMV colitis in immunosuppressed patients due to chemotherapy and/or corticosteroids [24]; CMV reactivation triggered by immune-mediated colitis [25]; or mixed immune-mediated colitis with CMV superinfection. As a precaution, CMV-positive patients were treated with VALGANCICLOVIR prior to corticosteroid initiation, with favorable outcomes.

The strengths of this study include a detailed description of immune- and chemotherapy-related toxicities by organ system, integrating clinical, biological, pathological, and radiological data. This multidimensional approach, resulting from collaboration between oncologists, clinical immunologists and organ specialists, improves understanding of the expected toxicity profile and may assist clinicians in patient management. Our data show that the frequency of grades 3–4 toxicities is broadly comparable to that in KEYNOTE-522, and that these adverse events are, in most cases, manageable. Finally, we observed encouraging efficacy signals in our population, with a pCR rate comparable to that in KEYNOTE-522 (61.1% vs. 63%) [24] and a 100% overall survival rate at 30 months among patients achieving complete pathological response.

The limitations of this study include its retrospective design, which is subject to inherent biases (missing data, heterogeneity of reporting) and the potential underestimation of some clinical or laboratory toxicities due to the lack of systematic documentation. Moreover, attributing certain toxicities specifically to immunotherapy or chemotherapy was sometimes challenging (e.g., diarrhea, transaminase elevation, troponin elevation), particularly for grades 1–2 events, where limited diagnostic work-up is performed usually—hence our focus on grades 3–4 toxicities for interpretation. Another limitation is the relatively short median follow-up (22 months), which precludes a robust analysis of predictive factors for recurrence-free survival or overall survival; these will be addressed in a subsequent publication after a longer follow-up.

Key Messages/Conclusion

- Grades 3–4 toxicities occurred in 20% of patients treated with neoadjuvant chemotherapy plus pembrolizumab for localized TNBC in our real-world practice.
- Patients with grades 3–4 irAEs tended to have higher pCR rates (80% vs. 56.8%; $p = 0.079$).
- The number of neoadjuvant pembrolizumab cycles did not influence the pCR rate.
- 16.3% of the cohort exhibited elevated troponin levels, 2% had documented immune-related myocarditis.

- Postoperative troponin elevation could be related to MINS; however, immune-related myocarditis should be ruled out first.
- Baseline ANA status does not appear to have predictive or prognostic value for severe toxicity occurrence.

These results are generally reassuring, especially as pembrolizumab is currently being investigated in other breast cancer subtypes, including hormone receptor-positive disease in the neoadjuvant setting, with promising pCR results from KEYNOTE-756 [25]. Survival data from this trial are awaited

Ethical approval: Ethical approval was obtained from the Research and Ethics Committee at Montpellier Cancer Center.

Patients consent: Patients were informed of the potential reuse of their clinical data through a general information notice and an objection form allowing them to decline. None chose to object.

Availability of data and material: Data are available from the corresponding author upon reasonable request.

Competing interests: All authors declare that they have no conflicts of interest regarding the publication of this article.

Funding: No funding was received for conducting this analysis.

Authors' contributions: Study design: IBA and PW. Manuscript drafting: IBA and PW. Critical review: Xavier Quatin, William Jacot, and Severin Guiu.

Acknowledgments: None.

References

1. Panorama des cancers en France – Édition 2024 [Internet]. [cité 20 avr 2025]. Disponible sur: <https://www.cancer.fr/catalogue-des-publications/panorama-des-cancers-en-france-edition-2024>
2. Ter Brugge P, Moser SC, Bièche I, Kristel P, Ibadioune S, Eeckhoutte A, et al. Homologous recombination deficiency derived from whole-genome sequencing predicts platinum response in triple-negative breast cancers. *Nat Commun*. 7 avr 2023;14(1):1958.
3. Serrano García L, Jávega B, Llombart Cussac A, Gión M, Pérez-García JM, Cortés J, et al. Patterns of immune evasion in triple-negative breast cancer and new potential therapeutic targets: a review. *Front Immunol*. 2024;15:1513421.
4. Gennari A, André F, Barrios CH, Cortés J, Azambuja E de, DeMichele A, et al. ESMO Clinical Practice Guideline for the diagnosis, staging and treatment of patients with metastatic breast cancer☆. *Ann Oncol*. 1 déc 2021;32(12):1475-95.
5. Pembrolizumab in Early-Stage Triple-Negative Breast Cancer. *N Engl J Med*. 12 mars 2025;392(11):1140-3.
6. Haute Autorité de Santé [Internet]. [cité 14 sept 2025]. KEYTRUDA (pembrolizumab) - Cancer du sein. Disponible sur: https://www.has-sante.fr/jcms/p_3325988/fr/keytruda-pembrolizumab-cancer-du-sein
7. PORTAIL AP KEYTRUDA — MSD [Internet]. [cité 20 avr 2025]. Disponible sur: <https://www.apkeytruda.fr/portail/>
8. Pembrolizumab in Early-Stage Triple-Negative Breast Cancer. *N Engl J Med*. 12 mars 2025;392(11):1140-3.
9. Freitas-Martinez A, Santana N, Arias-Santiago S, Viera A. Using the Common Terminology Criteria for Adverse Events (CTCAE - Version 5.0) to Evaluate the Severity of Adverse Events of Anticancer Therapies. *Actas Dermosifiliogr*. janv 2021;112(1):90-2.
10. Luetkens JA, Faron A, Isaak A, Dabir D, Kuetting D, Feisst A, et al. Comparison of Original and 2018 Lake Louise Criteria for Diagnosis of Acute Myocarditis: Results of a Validation Cohort. *Radiol Cardiothorac Imaging*. 25 juill 2019;1(3):e190010.

11. KEYNOTE-522 - Adverse Reactions & Safety Data [Internet]. [cité 26 mai 2025]. Disponible sur: https://www.keytrudahcp.com/safety/adverse-reactions/early-stage-triple-negative-breast-cancer/?utm_source=chatgpt.com
12. Rached L, Peyre-Pradat F, Spotti M, Baldini C, Laparra A, Lambotte O, et al. Real-world safety and effectiveness of neoadjuvant chemotherapy combination with pembrolizumab in triple-negative breast cancer. *ESMO Real World Data Digit Oncol*. 1 sept 2024;5:100061.
13. Kulkarni A, Marcucci M, Nguyen JMV. Myocardial injury after noncardiac surgery. *CMAJ*. 21 mai 2024;196(19):E661-E661.
14. American College of Cardiology [Internet]. [cité 26 mai 2025]. Combination Chemotherapy/Immunotherapy Regimens in Patients With Breast Cancer. Disponible sur: https://www.acc.org/Latest-in-Cardiology/Articles/2024/07/02/10/33/http%3a%2f%2fwww.acc.org%2fLatest-in-Cardiology%2fArticles%2f2024%2f07%2f02%2f10%2f33%2fCombination-Chemotherapy-Immunotherapy-Regimens-in-Patients-With-Breast-Cancer%3futm_source%3dchatgpt.com
15. Zhou F, Liu G, Zhang S, Luo C, Hu S, Wan S, et al. Cardiotoxicity in cancer immunotherapy: a systematic review and global meta-analysis. *J Transl Med*. 1 juill 2025;23:718.
16. Schmid P, Cortes J, Puztai L, McArthur H, Kümmel S, Bergh J, et al. Pembrolizumab for Early Triple-Negative Breast Cancer. *N Engl J Med*. 27 févr 2020;382(9):810-21.
17. Hountondji L, Ferreira De Matos C, Lebossé F, Quantin X, Lesage C, Palassin P, et al. Clinical pattern of checkpoint inhibitor-induced liver injury in a multicentre cohort. *JHEP Rep*. juin 2023;5(6):100719.
18. Ghosh N, Chan KK, Jivanelli B, Bass AR. Autoantibodies in Patients With Immune-Related Adverse Events From Checkpoint Inhibitors: A Systematic Literature Review. *J Clin Rheumatol Pract Rep Rheum Musculoskelet Dis*. 1 mars 2022;28(2):e498-505.
19. Hountondji L, Faure S, Palassin P, Viel PWD, Dupuy M, Larrey D, et al. Time to use the right classification to predict the severity of checkpoint inhibitor-induced liver injury, as assessed for causality using the updated RUCAM. *Aliment Pharmacol Ther*. déc 2024;60(11-12):1561-72.
20. Schmid P, Cortes J, Dent R, McArthur H, Puztai L, Kümmel S, et al. Overall Survival with Pembrolizumab in Early-Stage Triple-Negative Breast Cancer. *N Engl J Med*. 28 nov 2024;391(21):1981-91.
21. Belum VR, Benhuri B, Postow MA, Hellmann MD, Lesokhin AM, Segal NH, et al. Characterisation and management of dermatologic adverse events to agents targeting the PD-1 receptor. *Eur J Cancer Oxf Engl* 1990. juin 2016;60:12-25.
22. Kennedy OJ, Ali N, Lee R, Monaghan P, Adam S, Cooksley T, et al. Thyroid dysfunction after immune checkpoint inhibitors in a single-centre UK pan-cancer cohort: A retrospective study. *Eur J Cancer* [Internet]. 1 mai 2024 [cité 12 mai 2025];202. Disponible sur: https://www.ejancer.com/article/S0959-8049%2824%2900125-4/fulltext?utm_source=chatgpt.com
23. Osorio JC, Ni A, Chaft JE, Pollina R, Kasler MK, Stephens D, et al. Antibody-mediated thyroid dysfunction during T-cell checkpoint blockade in patients with non-small-cell lung cancer. *Ann Oncol*. 1 mars 2017;28(3):583-9.
24. Neven P, Floris G, Desmedt C. Pembrolizumab in Early-Stage Triple-Negative Breast Cancer. *N Engl J Med*. 13 mars 2025;392(11):1140-1.
25. Cardoso F, O'Shaughnessy J, Liu Z, McArthur H, Schmid P, Cortes J, et al. Pembrolizumab and chemotherapy in high-risk, early-stage, ER+/HER2- breast cancer: a randomized phase 3 trial. *Nat Med*. févr 2025;31(2):442-8.

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.