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ORIGINAL RESEARCH

Results of a phase I–II study of adjuvant concurrent carboplatin and accelerated radiotherapy for triple negative breast cancer

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ABSTRACT

Purpose: To determine feasibility and explore the clinical efficacy of concurrent radiotherapy and carboplatin as adjuvant treatment of triple negative breast cancer (TNBC).

Patients and Methods: Women with Stage I-II TNBC were treated after surgery in a phase I–II prospective trial [NCT01289353]. Weekly carboplatin (AUC = 2.0) was delivered for 6 weeks. Concurrent radiotherapy was delivered in the prone position during weeks 2–4, for a total dose of 40.5 Gy in 15 fractions to the breast, and 46.5 Gy in 17 fractions to the tumor bed. Adverse events (AE) were assessed weekly during treatment, once at 45–60 d, and every 6 mo thereafter, using the Common Terminology Criteria for AE (CTCAE) v3.0.

Results: A total of 39 patients accrued and 36 received treatment. Eight patients (22%, exact 95% CI: 10%, 39%) developed grade 2 or greater acute radiation dermatitis. Overall, grade 2 AE were seen in nine and grade 3 in two patients. Twenty-three patients (64%) received additional adjuvant chemotherapy. With a median follow-up of 48 mo, 34/36 (94%) are alive and disease free. One patient died of pulmonary failure with possible but unproven breast cancer recurrence, and one patient died of pelvic malignancy. One patient recurred locally and is alive and disease free after surgical management. Brisk lymphocytic infiltrate was present pre-treatment in 39% of 18 patients with evaluable tumor.

Conclusions: Adjuvant concurrent carboplatin and prone accelerated radiotherapy is a well-tolerated and promising treatment of early stage TNBC. The observed 3% compares favorably with the expected 30% recurrence rate within 1–4 y from treatment, warranting further studies.

Abbreviations: AE, adverse events; BC, breast cancer; CTCAE, Common Terminology Criteria for AE; DAMP, damage-associated molecular pattern; ICD, immunogenic cell death; LPBC, lymphocyte-predominant BC; pCR, pathological complete response; PDL-1, programmed death ligand-1; pPR, pathological partial response; TILs, tumor-infiltrating lymphocytes; TLR, toll-like receptor; TNBC, triple negative breast cancer

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Introduction

The contribution of standard cancer therapies such as chemotherapy and radiotherapy to the induction of a type of cell death that is sensed by the immune system as immunogenic was originally described in a series of reports from Laurence Zitvogel and Guido Kroemer's laboratories.[1-5] Their work identified three critical molecular signals that were required to induce an immunogenic cell death (ICD): translocation of calreticulin to the cell membrane to deliver an "eat me" signal,[2] release of HMGB-1, a damage-associated molecular pattern (DAMP) that binds to toll-like receptor-4 (TLR4) to promote cross-presentation of tumor-derived antigens,[4] and Adenosine triphosphate (ATP) released by dying cells that binds to P2RX7 purinergic receptor leading to inflammasome activation and IL-1β production.[5]

We developed an *in vitro* assay to expedite the testing of different combinations of chemotherapy and radiotherapy, to select those that best achieve ICD. The assay confirmed the dose-dependent effect of radiotherapy, for each of the three component of ICD. In addition, the combination of carboplatin and radiation was found to be a potent inducer of ICD.[6] This finding led to the hypothesis that *in vivo* carboplatin and concurrent radiotherapy may trigger an adaptive antitumor immune response. This background inspired the design of a phase I–II clinical trial (NCT01289353) to test feasibility and explore clinical efficacy of this combination in the adjuvant setting of triple negative breast cancer (TNBC).

The rationale for combining radiation with concurrent carboplatin was also based on the specific vulnerability of TNBC. Several studies postulated that *BRCA* gene inactivation might

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also have a role in sporadic TNBCs and have defined as "*BRCA*-ness" the multiplicity of DNA repair deficiencies associated with these tumors.[7-9] Repair deficiencies of TNBC can be exploited by treatment with platinum salts, cisplatin, or carboplatin.[10-12] Platinum agents cause covalent crosslinks within the DNA double helix and interfere with the progression of the replication fork. In a setting of defective BRCA1 and BRCA2, breast cancer cells either attempt to repair the damaged DNA by mechanisms like non-homologous end joining further acquiring genomic instability, or fail to repair the DNA damage caused by platinum agents, resulting in programmed cell death.[9] An analogous vulnerability to ionizing radiation among *BRCA* mutation carriers and in tumors with *BRCA*-ness has been established.[12,13]

In other tumor types than breast cancer, concurrent chemoradiation with a platinum compound and radiotherapy has demonstrated efficacy and superiority to a sequential approach.[14,15] In this study, we used the prone accelerated radiotherapy regimen, delivered over 3 weeks that we have extensively tested in the adjuvant setting of breast cancer,[1617] and slightly modify it to isolate the tumor bed boost on two Sundays during the 3 weeks, to reduce the risk of skin toxicity when radiation was used with a powerful radio-sensitizer such as carboplatin.

Moreover, because of the promising clinical results at 48 mo of median follow-up, we quantified tumor-infiltrating lymphocytes (TILs) in patients who had available material from surgical specimens. TILs are a parameter that has been shown to have prognostic value in TNBC patients treated with adjuvant anthracycline-based chemotherapy.[18,19] Interestingly, in the neo-adjuvant GeparSixto trial, patients with TN and HER-2+ tumors with >60% stromal TILs had 3.71-fold increase in the odds of pathological complete response (pCR) if they had received carboplatin.[20] These results support the hypothesis that carboplatin has a significant interaction with the immune system.

The results of NCT01289353 are reported. The trial demonstrates the optimal feasibility of the combination and preliminary efficacy at a median follow-up of 4 y, confirming the translation to the clinic of preclinical predictions.

Results

Patient characteristics

Thirty-nine patients signed an informed consent to participate to the trial. Three patients elected to withdraw consent before initiation of any treatment, and 36 patients completed the trial as designed and were evaluable. Among these, 36 patients 61% were older than 50 (age median 55.5 y, range 27–82 y), 44.3% had tumors larger than 2 cm in diameter, and 16% were node positive (Table 1).

Feasibility

Only two patients (5.5%) had grade 3 acute AE (one wet desquamation and one pain), demonstrating the feasibility of the concurrent administration of carboplatin and radiation used.

Table 1. Baseline patient characteristics ($n = 36$).

Variable		Frequency	Percentage
Age (years)	<50	13	36.1
	≥ 50	23	63.9
Race	Asian	5	13.9
	Black	8	22.2
	Other	3	8.3
	White	20	55.6
Hispanic status	Hispanic	2	5.6
	Non-Hispanic	31	86.1
	Unknown	3	8.3
Lymph node metastasis	Positive	6	16.7
	Negative	30	83.3
Tumor size	R T1a; L T1b	1	2.8
	R T2; L T3	1	2.8
	T1a	4	11.1
	T1b	5	13.9
	T1c	9	25.0
	T1mi	1	2.8
	T2	15	41.7
Tumor grade	III	34	94.4
	Unknown	2	5.6
LVI	Positive	3	8.3
	Negative	28	77.8
	Unknown	5	13.9
Additional chemotherapy	AC	1	2.78
	CMF	4	11.11
	TC	3	8.33
	Abraxane	1	2.8
	ddAC-T	13	36.1
	Doxil	1	2.8
	None	13	36.11

Safety

The primary end point of the study was the occurrence of grade 2 or greater acute carbo-radiation toxicity. Eight patients (22%, exact 95% CI: 10%, 39%) developed grade 2 or greater acute radiation dermatitis (Table 2). Grade 2–3 pain occurred in two patients (5.6%) and grade 2 fatigue in one patient (2.8%). All acute toxicities resolved within 60 d from inception of combined chemoradiation. Although the trial end point was limited to acute toxicity, late effects of the combined carboplatin and radiation were acceptable, without grade 3–4 toxicity detected at last follow-up.

Efficacy

At a median follow-up of 4 y, one patient has died from fulminant pulmonary failure and thrombotic angiopathy 5.6 mo from trial completion. As there was no biopsy or autopsy-proven tumor, a breast cancer recurrence could not be excluded. A second patient developed an in-breast recurrence, contiguous to the original tumor bed, 27 mo after treatment. She underwent salvage mastectomy and is alive without evidence 47 mo from diagnosis. One patient

Table 2. Maximum grade acute carboplatin-radiation AE ($n = 36$).

AE	Grade 1	Grade 2	Grade 3	Total
Wet desquamation	0	3	1	4
Erythema	27	4	0	31
Pain	7	1	1	9
Fatigue	15	1	0	16
Edema	4	0	0	4
Dry desquamation	9	0	0	9

Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Additional Adjuvant Chemo
C	C/RT	C/RT	C/RT	C	C	

WEEK	WEEK 2						WEEK 3							WEEK 4					
DAY #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
	M	T	W	T	F		S	M	T	W	T	F		S	M	T	W	T	F
Tx	wb*	wb	wb	wb	wb		wb	wb	wb	wb	wb	B+		wb	wb	wb	wb	wb	B+

*wb = target is whole breast, 2.7 Gy/fraction
+B = boost to tumor bed 3 Gy (second and third Friday)

Figure 1. Treatment schema. The trial consisted of 6 weeks of weekly carboplatin (AUC = 2) delivered with concurrent breast radiotherapy (top panel), during week 2–4, as detailed in the bottom panel. Additional adjuvant chemotherapy was administered at the discretion of the treating medical oncologist, for the patients who accepted additional treatment.

succumbed to disseminated pelvic malignancy more than 2 y after the completion of treatment. The remainder 33 patients are alive and have remained disease free (Fig. 2).

Analysis of the tumor lymphocytic infiltrate and PDL-1 expression

Eighteen patients had original tumor slides available for evaluation. No significant differences were observed with respect to baseline and clinical characteristics between patients with available tumor slides and patients without available slides (Table S1). Total 7/18 tumors showed >60% stromal TILs, fulfilling the definition of lymphocyte-predominant BC (LPBC)[20] (Table 3 and Fig. 3). One patient had tertiary lymphoid structures (TLS) adjacent to the tumor, a feature associated with improved outcome[21] (Fig. 3). Among the non-LPBC, 8/18 had >10% TILs, indicating that most patients had favorable prognostic characteristic.[18,19] Interestingly, the only patient with a proven recurrence had low TILs (Table 3).

For 11 of the 18 cases, sufficient tissue was available for the evaluation of programmed death ligand-1 (PDL-1) expression in the tumor. PDL-1 is a ligand for the immune-checkpoint receptor programmed death-1 (PD-1) expressed on T cells and represents a major mechanisms of immune escape and a therapeutic target in many tumors including breast cancer.[22,23] PDL-1 was expressed at significant levels in most of the tumors with high TILs (Table 3 and Fig. 3), consistent with the phenomenon of induced resistance whereby cancer cells upregulate PDL-1 in response to IFNγ produced by the infiltrating antitumor T cells.[24]

The neutrophil-to-lymphocyte ratio (NLR) was analyzed for this cohort. None of the 36 patients had a baseline ratio >4: The NLR mean value was 2.19 (range 0.93–3.76). A mixed effects longitudinal analysis of neutrophil and lymphocyte levels in peripheral blood over time was also conducted for all patients. Neutrophil levels decreased significantly during treatment (Pre–During: −0.90 with 95% CI: −1.4, −0.42) and from pre-treatment to post-treatment (−1.27 with 95% CI: −1.78, −0.76) (Table S2). Similarly, lymphocyte levels decreased significantly during treatment (Pre–During: −0.54 with 95% CI: −0.69, −0.42) and from pre-treatment to post-treatment (−0.50 with 95% CI: −0.66, −0.34) (Table S3). Furthermore, longitudinal analyses were conducted to assess changes in the NLR over time. The ratio increased during treatment (Pre–During: 0.15 with 95% CI: −0.11, 0.41) and decreases from pre-treatment to post-treatment (−0.19 with 95% CI: −0.47, 0.09). Both of these changes were not significant (Table S4).

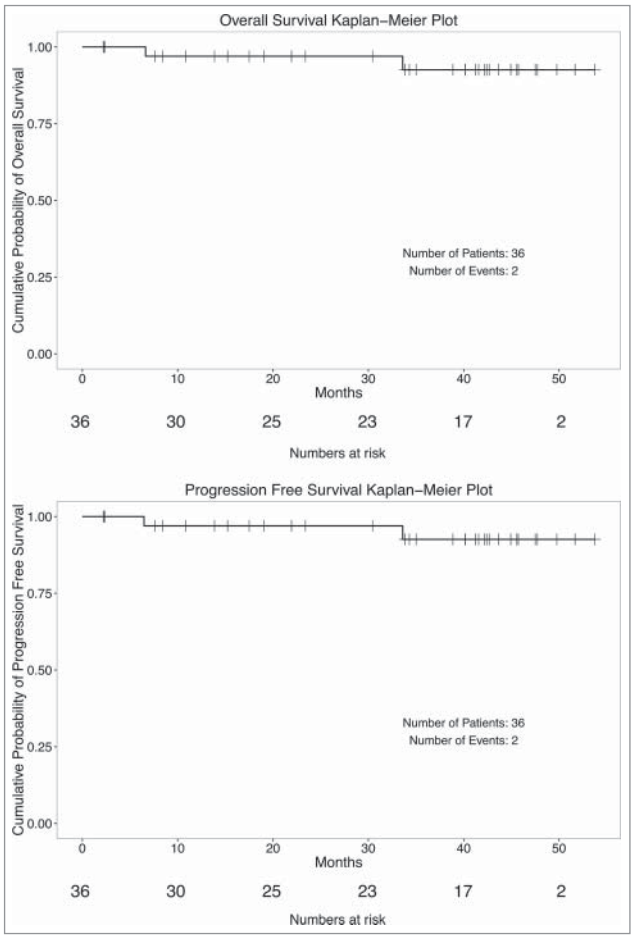

Figure 2. Kaplan–Meier plots of progression-free survival and disease-free survival (n = 36) of patients with TNBC treated with adjuvant concurrent carboplatin and accelerated radiotherapy.

Table 3. TILs infiltration and PDL-1 expression ($n = 18$).

Patient ID#	Stromal TILs (%)	PDL-1 expression
1	20	ND[a]
4	10	<1% (2+)
11	90	ND
13	50	ND
15	60	ND
18	40	5% (3+)
19	50	5% (1–2+)
23	60	50% (2–3+)
25	70	70% (1–3+)
28	30	ND
29	10	<1% (1–2+)
32	80[b]	30% (1–3+)
33	10	0
35[c]	Left: 30; Right: 5	Left: 10% (2–3+); Right: <1%
36[d]	10	0
37	70	1% (1–2+)
38	30	10% (2–3+)
39	80	50% (1–3+)

[a]ND, not done.
[b]Tertiary lymphoid structures (TLS) were present adjacent to the tumor.
[c]This patient had bilateral TNBC and both tumors were evaluated.
[d]Patient experiencing a local tumor recurrence.

Additional systemic chemotherapy

A total of 23 of 36 patients (64%) received some additional systemic chemotherapy, after concurrent carboplatin and radiation. Specifically, 13 patients (36%) underwent dose dense ACT (four cycles of doxorubicin and cyclophosphamide followed by paclitaxel every 2 weeks), and four patients underwent CMF (cyclophosphamide, methotrexate, and 5fluorouracil, every 3 weeks for eight cycles), three patients received docetaxel and cyclophosphamide every 3 weeks for four cycles, one patient received AC (doxorubicin and cyclophosphamide for four cycles), one patient received nab-paclitaxel for five cycles, and one patient received liposomal doxorubicin for four cycles.

Discussion

TNBC characterizes an aggressive group of breast cancers with a high risk of distant recurrence and death after initial surgical treatment. In a seminal paper that describes the natural history of the different subtypes of breast cancer, Dent et al. demonstrated the increased risk of death from disease among TNBC patients (42.2%) in the period between 1 and 4 y from diagnosis when compared to hormone receptors-positive and/or HER2-positive breast cancer, and a sharp decline of the recurrence rate of TNBC after 5 y of follow-up.[25] At a median follow-up of 4 y, only two patients (5.3%) in the current study have died, one without confirmed recurrent disease and another with a metastatic pelvic malignancy. Only one patient developed an isolated in breast recurrence, contiguous to the original tumor bed, and she is alive and free of disease after surgical management of the recurrent tumor. The meaning of a local recurrence in TNBC is different from that of other breast cancer subtypes. In the original series from Dent et al., a local recurrence heralded distal recurrence in 25% of TNBC carriers versus 44% of the patients with the other subtypes of breast cancer ($p = 0.02$).[25]

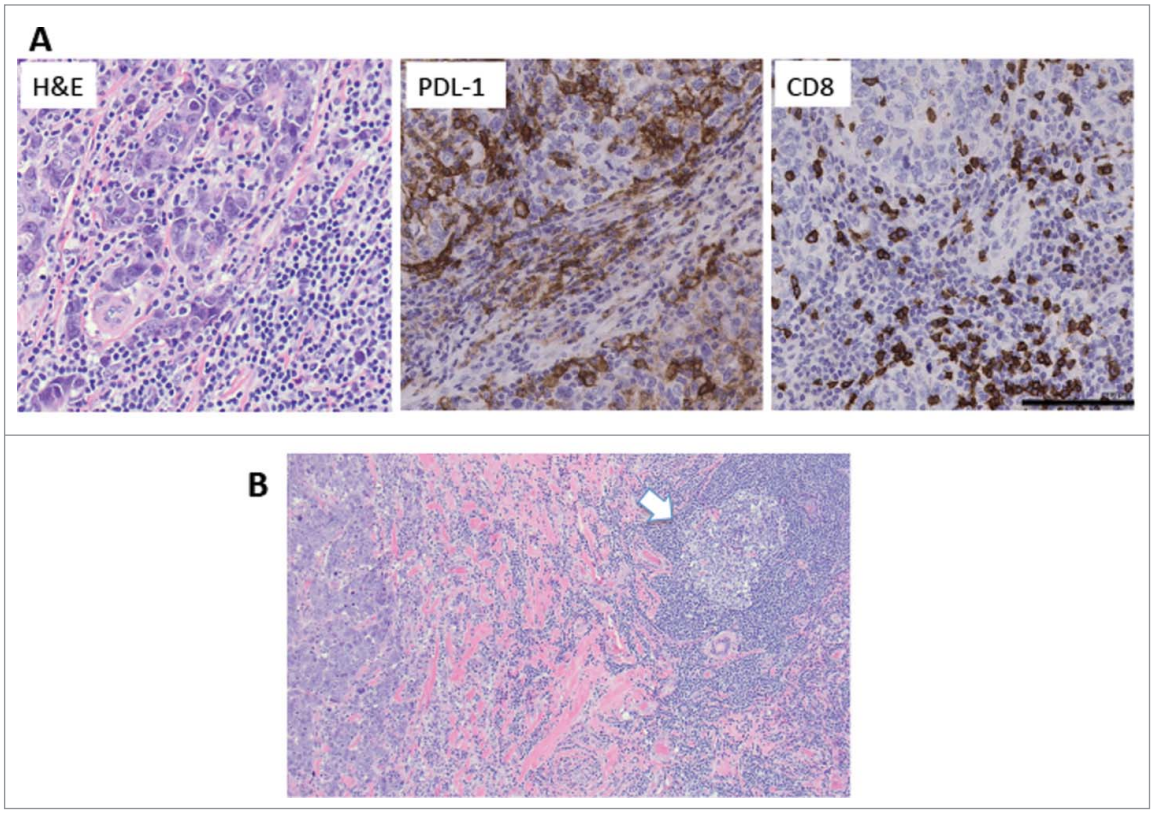

Figure 3. TILs, PDL-1 expression, $CD8^+$ distribution and TLS in the tumor of patient ID#32. (A) Example of tumor with 80% stromal TILs, as assessed on H&E sections. The tumor showed strong expression of PDL-1 on the cancer cells and infiltrating immune cells, and $CD8^+$ T cells infiltrating tumor cell nests. Magnification 200X, bar = 100 μm. (B) TLS (arrow) was present at the periphery of the tumor. Magnification 100X.

Inclusion of radiotherapy to the breast in the management of TNBC who had undergone breast conservation was associated with statistically significant increase in survival in a retrospective analysis of 249 patients from Washington University.[26] The adjuvant regimen of concurrent carboplatin and radiotherapy tested in this study was chosen to translate to the clinic the pre-clinical findings of increased ICD of the combination of carboplatin and ionizing radiation.[6] The concurrent administration of carboplatin and accelerated radiotherapy was well tolerated with no increase in acute radiation dermatitis above the baseline expected from radiation alone, and only 2/36 (5.6%) patients with any grade 3 AE, demonstrating feasibility and safety of this treatment.

Previous experience in the neo-adjuvant setting of locally advanced breast cancer both with continuous infusion 5Fluorouracil[27] and with twice weekly paclitaxel[28] supports the benefit of the use of concurrent chemo-radiation for TNBC. The latter combination was tested at three institutions, USC, NYU, and Vanderbilt in 105 patients with locally advanced breast cancer treated with paclitaxel (30 mg/m^2 intravenously twice a week) for 10–12 weeks[28] with daily breast and nodal radiotherapy during weeks 2–7. Pathological response (pCR and pPR) after neoadjuvant chemo-radiation was achieved in 36/105 patients (34%, 95% CI: 25–44%). TNBC patients had a 54.2% pathological response rate. Patients with pathologic response had a lower risk of recurrence or death compared with non-responders (hazard ratio = 0.35, 95% CI: 0.15–0.80, log-rank p-value = 0.01).[29] In a companion study conducted in the same population to identify molecular markers of pathologic response to neoadjuvant paclitaxel/radiation treatment by protein and gene expression profiling performed on pretreatment biopsies, a significant enrichment in immune-related gene was found in patients with pCR.[30]

Thus, based on this clinical experience and growing experimental evidence that the therapeutic success of cytotoxic treatments relies on their ability to induce antitumor immune responses,[31] we originally hypothesized that the success of concurrent chemo-radiation may derive from the induction of ICD by the combination.[32,33] The adjuvant setting of TNBC offered the opportunity for testing this approach in a clinical situation of minimal tumor burden, ideal for the success of an immune response, and in a disease with generally shorter median time to recurrence.

The role of TILs as predictors of outcome in TNBC has been demonstrated in several adjuvant and neo-adjuvant studies.[18-20,34] In this study, we were able to retrieve the original tumor specimen and evaluate TILs in only half of the patients. This analysis showed that in 39% of the patients tested, the tumor was associated with >60% TIL infiltrate. This type of TIL-rich breast cancer has been designated as LPBC.[34] In a study in which 28.3% of 314 TNBC and 19.9% of 266 HER2+ tumors were LPBC, addition of carboplatin to neoadjuvant chemotherapy increased the odds of pCR 3.71-fold in LPBC, but only 1.01-fold in non-LPBC, suggesting a strong interaction of carboplatin with the immune system.[20] Because of the generalized good outcome of our cohort of patients at the current median follow-up of 4 y and the fact that in only half we could analyze the pretreatment tumor tissue, it is impossible to demonstrate whether with the degree of TILs infiltration correlates with outcome. Interestingly, the only patient in this series who recurred locally had low TIL infiltrate in the original segmental mastectomy specimen.

A recent re-classification of TNBC by Lehmann et al. has demonstrated the heterogeneity of this subset of breast cancer and introduced opportunities to identify distinct and specific therapeutic targets. Gene expression of 587 TNBC cases from 21 breast cancer data sets was conducted. Based on distinct gene ontology, six subtypes were identified and, pre-clinically, this genetic classification informed selection of therapeutic modalities.[35] No genetic analysis of the original tumor was conducted in the current study, but it is possible that the cohort of patients that accrued to this trial was enriched for the Basal-like group. This group encompasses the BL1 and BL2 subtypes that are enriched in the activation cell cycle and cell division and the DNA damage response pathways. Future studies should characterize TNBC to diversify its therapeutic approach and should further test the role of concurrent platinum compounds with radiation for the basal-like group.

In conclusion, this Phase I–II study of concurrent carboplatin and radiotherapy in the adjuvant setting of TNBC resulted in optimal tolerance and lack of significant alopecia with an excellent outcome at a median follow-up of 4 y, despite the fact that 36% of the patients in this series did not receive any additional adjuvant chemotherapy. It is intriguing to hypothesize that in a subset of TNBC a brief regimen of chemo-radiation may be recruiting an adaptive immune response producing a long-lasting immunological equilibrium and impacting disease-free survival. Testing this combination in a pre-surgical setting could enable a better understanding of the effects on the tumor and elucidate the role of different TNBC subtypes in the response to carboplatin and radiation.

Patients and methods

Patient characteristics

Patients with newly diagnosed Stage I–II (pT1-T2, pN0, and N1) TNBC were eligible to be enrolled into this clinical study, if they had refused or were not prescribed standard adjuvant chemotherapy (dose-dense ACT or TC), after breast cancer surgery (segmental mastectomy or mastectomy). Triple-negative status was defined at the time of study entry as estrogen and progesterone receptor expression in less than 1% of the cancer cells, according to the American Society of Clinical Oncology (ASCO)/College of American pathologists (CAP) guidelines[36] and HER2-negative at HercepTest [Dako] score 0 or 1+ or the gene amplification ratio <2·2 by *in situ* hybridization. The study was approved by NYU Institutional Review Board and was conducted in compliance with the Declaration of Helsinki. Clinical trial registration number is NCT01289353. Written informed consent was obtained from all patients.

Study design

This was a phase I–II single institution study. Weekly carboplatin (AUC = 2.0) was delivered for 6 weeks, with RT to the whole breast concurrently delivered during weeks 2–4, via a 3D-CRT or IMRT technique, in the prone position, as previously reported.[16,17] A total dose to the breast of 40.5 Gy was delivered in 15 fractions (Monday–Friday). The tumor bed received a boost of two additional fractions of 3 Gy, delivered

on two consecutive Sundays (before the 2nd and 3rd weeks of RT), for a total dose of 46.5 Gy to the tumor bed in 17 fractions over 19 d (Fig. 1). Additional adjuvant chemotherapy was administered at the discretion of the treating medical oncologist.

Feasibility, safety, and efficacy

This trial was designed to test the feasibility of the combined regimen, defined as <10% Grade 3 acute AE assessed within 60 d from the initiation of concurrent carboplatin and radiation. Exploratory endpoints were local and systemic recurrence. Safety of the regimen was defined as grade 2 or greater acute radiation dermatitis. AE were assessed weekly during treatment, and once at 45–60 d, using the Common Terminology Criteria for Adverse Events (CTCAE) v3.0. Patients were followed up every 6 mo after completion of treatment of local and systemic recurrence.

Evaluation of tumor-infiltrating lymphocytes (TILs), and Programmed cell death 1 ligand 1 (PDL-1) expression

TIL evaluation was performed on a single full-face hematoxylin and eosin (H&E)-stained section, which was available from 18 of the 36 patients who completed treatment. The percentage of stromal TILs was estimated by a pathologist with experience in this methodology (SD) following the consensus guidelines published by the TIL working group.[37] Results are reported as described previously[19] with small modifications, in increments of 10 for values of 10% or above; <1% is considered 0, and values >1 but <10% were rounded up to 5%. Patients with tumors with >60% stromal TILs were defined as LPBC.[20]

Tumor tissue was analyzed for PD-L1 expression by immunohistochemistry (IHC), performed on formalin-fixed, paraffin-embedded, 4-μm tissue sections using unconjugated, rabbit anti-human PDL-1 (CD274) clone SP142 (PDL1, Spring Biosciences catalog number M4420).[38] Staining was performed on a Ventana Medical Systems Discovery XT instrument with online deparaffinization and using Ventana's reagents and detection kits. PDL1 was antigen retrieved in Ventana Cell Conditioner 1 (Tris-Borate-EDTA) for 20 min. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide for 4 min. PDL1 was diluted 1:50 in phosphate buffered saline and incubated for 60 min at 37 °C. Primary antibody was detected with hapten linked, anti-rabbit multimer incubated for 20 min followed by anti-hapten horseradish peroxidase conjugate for 20 min. The complex was visualized with 3,3 diaminobenzidene and enhanced with copper sulfate. A tissue microarray containing placental tissue was used as positive control. PDL-1 expression was quantified as a percentage of tumor cells with positive membrane staining and intensity classified on a scale of 1–3. In some samples, $CD8^+$ T cell infiltration was assessed by staining with rabbit anti-human $CD8^+$ clone SP57 (Ventana Medical Systems catalog number 790–4460).

Statistical analysis

The primary objective of this trial was to estimate the proportion of patients with grade 2 or greater acute radiation dermatitis. A difference of +/− 18% could be detected (from a baseline rate of 25% of patients with grade 2 or greater acute radiation dermatitis) with a two-sided $\alpha = 0.05$ and power of 80% using an exact binomial test. If 15 or more events among these 37 patients were observed, the null hypothesis that the rate is 25% was to be rejected (Calculations from PASS 2008, NCSS).

Patient demographic and disease characteristics are summarized using frequency distributions for categorical variables and summary statistics and graphical displays for quantitative variables, including TILs and PDL-1 expression. To examine the changes in circulating leukocytes over time, we used a mixed effects longitudinal regression models for neutrophils (N) and lymphocytes (L) separately, and the N/L ratio in which we considered both the within patient and between patient variability.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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Author contributions

Authors SCF, YN, JDG, and SD contributed to conception and design; SCF, SD, JDG, and YN to the development of methodology; SCF, YN, MFK, JT, SD, SC, and JS to acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.); SCF, EBG, SD, YN, XL, and JDG to analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis); SCF, SD, YN, XL, and JDG to writing, review, and/or revision of the manuscript; and SCF, SC, MFK, and XL to administrative, technical, or material support (i.e., reporting or organizing data, constructing databases).

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