A Multicenter Phase II Trial of Ipilimumab and Nivolumab in Unresectable or Metastatic Metaplastic Breast Cancer: Cohort 36 of Dual Anti–CTLA-4 and Anti–PD-1 Blockade in Rare Tumors (DART, SWOG S1609)

Sylvia Adams[1], Megan Othus[2,3], Sandip Pravin Patel[4], Kathy D. Miller[5], Rashmi Chugh[6], Scott M. Schuetze[6], Mary D. Chamberlin[7], Barbara J. Haley[8], Anna Maria V. Storniolo[5], Mridula P. Reddy[9], Scott A. Anderson[10], Collin T. Zimmerman[11], Anne P. O'Dea[12], Hamid R. Mirshahidi[13], Jordi Rodon Ahnert[14], Frank J. Brescia[15], Olwen Hahn[16], Jane M. Raymond[17], David D. Biggs[18], Roisin M. Connolly[19], Elad Sharon[20], Larissa A. Korde[20], Robert J. Gray[21], Edward Mayerson[2,3], Melissa Plets[2,3], Charles D. Blanke[22], Young Kwang Chae[23], and Razelle Kurzrock[4]

ABSTRACT

Purpose: Metaplastic breast cancer (MpBC) is a rare aggressive subtype that responds poorly to cytotoxics. Median survival is approximately 8 months for metastatic disease. We report results for advanced MpBC treated with ipilimumab + nivolumab, a cohort of S1609 for rare cancers (DART: NCT02834013).

Patients and Methods: Prospective, open-label, multicenter phase II (two-stage) trial of ipilimumab (1 mg/kg i.v. every 6 weeks) plus nivolumab (240 mg i.v. every 2 weeks) for advanced MpBC. Primary endpoint was objective response rate (ORR). Secondary endpoints included progression-free survival (PFS), overall survival (OS), and toxicity.

Results: Overall, 17 evaluable patients enrolled. Median age was 60 years (26–85); median number of prior therapy lines was 2 (0–5). ORR was 18%; 3 of 17 patients achieved objective responses (1 complete, 2 partial responses; 2 spindle cell, 1 chondromyxoid histology), which are ongoing at 28+, 33+, and 34+ months, respectively. Median PFS and OS were 2 and 12 months, respectively. Altogether, 11 patients (65%) experienced adverse events (AE), including one grade 5 AE. Eight patients (47%) developed an immune-related AE (irAE), with adrenal insufficiency observed in all 3 responders. Responses occurred in tumors with low tumor mutational burden, low PD-L1, and absent tumor-infiltrating lymphocytes.

Conclusions: The ipilimumab and nivolumab combination showed no new safety signals and met its primary endpoint with 18% ORR in advanced, chemotherapy-refractory MpBC. All responses are ongoing at >2 to almost 3 years later. The effect of ipilimumab and nivolumab was associated with exceptional responses in a subset of patients versus no activity. This combination warrants further investigation in MpBC, with special attention to understanding mechanism of action, and carefully designed to weigh against the significant risks of irAEs.

Introduction

Metaplastic breast cancers (MpBC) are rare (~1% of breast cancers) and very aggressive tumors, typically composed of both an adenocarcinoma and a metaplastic component (squamous, chondroid, spindle, rhabdoid, or osseous, typically of same clonal origin as ductal carcinoma component; refs. 1–3). MpBC has a poor response to standard cytotoxic therapies (4), and a median survival of 8 months for metastatic disease, which is significantly worse than that of nonmetaplastic triple-negative breast cancer (TNBC; ref. 5).

The molecular signature of MpBCs has similarities to the claudin-low and mesenchymal subtypes of TNBC. There is an enrichment of

[1]New York University Perlmutter Cancer Center, NYU Langone Health, New York, New York. [2]SWOG Statistics and Data Management Center, Seattle, Washington. [3]Fred Hutchinson Cancer Research Center, Seattle, Washington. [4]University of California San Diego Moores Cancer Center, La Jolla, California. [5]Indiana University Melvin and Bren Simon Cancer Center, Indianapolis, Indiana. [6]University of Michigan, Ann Arbor, Michigan. [7]Dartmouth-Hitchcock Medical Center/Norris Cotton Cancer Center, Lebanon, New Hampshire. [8]UT Southwestern/Simmons Cancer Center-Dallas, Dallas, Texas. [9]Dayton Physicians LLC-Atrium Hematology Medical Oncology Division (Dayton NCORP), Franklin, Ohio. [10]Goldschmidt Cancer Center, Capital Region Southwest Campus (Heartland NCORP), Jefferson City, Missouri. [11]Kaiser Permanente Medical Group (Kaiser Perm NCORP), San Diego, California. [12]University of Kansas Hospital–Westwood Cancer Center (Kansas MU-NCORP), Westwood, Kansas. [13]Loma Linda University Cancer Center, Loma Linda, California. [14]MD Anderson Cancer Center, Houston, Texas. [15]MUSC, Hollings Cancer Center (MUSC MU-NCORP), Charleston, South Carolina. [16]University of Chicago Comprehensive Cancer Center, Chicago, Illinois. [17]Allegheny General Hospital, Pittsburgh, Pennsylvania. [18]Centura Health, Frisco, Colorado. [19]Johns Hopkins University/Sidney Kimmel Cancer Center, Baltimore, Maryland. [20]National Cancer Institute, Cancer Therapy Evaluation Program, Bethesda, Maryland. [21]Dana-Farber Cancer Institute-ECOG-ACRIN Biostatistics Center, Boston, Massachusetts. [22]SWOG Group Chair's Office, Knight Cancer Institute, Oregon Health and Science University, Portland, Oregon. [23]Northwestern University, Chicago, Illinois.

Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

Current address for R.M. Connolly: University College Cork, Cork, Ireland.

Trial Registration: ClinicalTrials.gov registry: NCT02834013.

Prior presentation: The study has been presented in part at the Annual ASCO Meeting in June 2020.

Corresponding Author: Sylvia Adams, NYU Langone Health, Perlmutter Cancer Center, New York, NY 10016. Phone: 212-731-5795, E-mail: sylvia.adams@nyulangone.org

Clin Cancer Res 2022;28:271–8

doi: 10.1158/1078-0432.CCR-21-2182

Translational Relevance

SWOG dual anti–CTLA-4 and anti–PD-1 blockade in rare tumors (DART) S1609 is the first study of combination anti–CTLA-4 (ipilimumab) and anti–PD-1 (nivolumab) across rare tumors, with this cohort focusing on metaplastic breast cancer, an aggressive subtype that responds poorly to cytotoxic therapies and in whom immunotherapies have not previously been evaluated. Patients with advanced, chemotherapy-refractory metaplastic breast carcinoma had an 18% objective response rate (3/17 patients), which may be driven in part by anti–CTLA-4 as part of the treatment combination because responders all had low tumor mutational burden and included tumors with low tumor-infiltrating lymphocytes and low PD-L1 expression. All responses are ongoing beyond 2+ years.

stem cell–associated genes including genes involved in epithelial-to-mesenchymal transition (6). Genomic studies have found that MpBC may have amplification of epidermal growth factor receptor, as well as alterations in genes involved in the PI3K/Akt pathway, Wnt/β-catenin signaling, and cell-cycle dysregulation (7).

Frequent overexpression of PD-L1 was recently demonstrated in primary MpBC, with PD-L1 positivity in tumor cells (8). Tumor-infiltrating lymphocytes (TIL) have also been demonstrated in MpBC, including PD-1–expressing TILs (8, 9), suggestive of an immunogenic cancer phenotype in some patients. These findings and preliminary evidence of clinical activity of PD-1 blockade in MpBC (10) led to the inclusion of MpBC into the DART study as cohort 36.

Here, we present the results of the metaplastic breast cancer cohort on DART (DUAL ANTI–CTLA-4 AND ANTI–PD-1 BLOCKADE IN RARE TUMORS, S1609), a prospective phase II study conducted through the National Cancer Institute (NCI)–supported SWOG Cancer Research Network.

Patients and Methods

Patients and procedures

DART is a multicenter (>800 sites), open label, phase II basket study (NCT02834013) of ipilimumab and nivolumab for rare malignancies. It is being conducted by the Early Therapeutics and Rare Cancers Committee under the auspices of SWOG and the NCI. The Cancer Therapy Evaluation Program (CTEP) provided study medication under an NCI Cooperative Research and Development Agreement (CRADA) with Bristol–Myers Squibb (BMS). The study was conducted in accordance with the Declaration of Helsinki. The trial design and eligibility criteria for DART were previously reported (11). All participants provided written informed consent authorized by each enrolling center's internal review board.

Eligible patients for cohort 36 must have had a histologically confirmed diagnosis of MpBC with disease measurable as per RECIST v1.1 (12). Enrollment was directed into study cohorts (baskets) based on the local pathology report (the DART trial did not mandate central review/verification of the rare histologies). All patients' cancers had progressed following at least one line of standard therapy and there must not have been other approved/standard therapy available that has been shown to prolong overall survival. Patients may have received either prior anti–CTLA-4 or prior anti–PD-1/anti–PD-L1 therapy (but not both), provided that it was completed at least 4 weeks prior to registration. Patients were required to have an Eastern Cooperative Oncology Group performance status (ECOG PS) 0–2, be at least 18 years of age, and have adequate organ function, within specific hematologic, renal, hepatic, adrenal, and thyroid parameters. Exclusion criteria included certain autoimmune diseases and ongoing grade 3/4 immune-related adverse events (irAE). For patients with brain metastases, central nervous system (CNS)-directed therapy must have been completed ≥28 days prior to registration and patients must have been off steroids for at least 7 days with stable disease at time of registration.

Patients received nivolumab 240 mg every 2 weeks and ipilimumab 1 mg/kg every 6 weeks (both intravenously, one cycle is 6 weeks). Disease assessments were performed at baseline and thereafter at weeks 8, 16, 24, and then every 12 weeks. Treatment continued until tumor progression, unacceptable toxicity, or withdrawal of consent.

Endpoints and statistical analysis

The primary endpoint of the study was objective response rate [ORR; confirmed complete and partial response (CR and PR, respectively)] as assessed by RECIST 1.1 criteria (12). The regimen was considered of interest for further study if the true ORR was 12% or higher (2 responses out of 16 eligible patients). Subset analyses within the cohort were not prespecified.

As previously described, all cohorts used a two-stage design (13). If ≥1 response was observed in the first 6 eligible and evaluable patients, accrual to the second stage to a total of 16 patients would be opened.

Table 1. Patient summary.

	N (%) or median (min, max)
Age	60 (26, 85)
Female sex	17 (100)
ECOG PS	
0	5 (29)
1	10 (59)
2	2 (12)
Race/Ethnicity	
White	14 (82)
Black	2 (12)
Asian	1 (6)
Hispanic	1 (6)
Biomarker profile	
ER/PR/HER2 neg (TNBC)	13 (76)
ER or PR low (1%–10% pos), HER2 neg	3 (18)
ER/PR > 10% pos, HER2 neg	1 (6)
Ki67	87 (20, 100)
Histology	
Spindle	8
Squamous	3
Spindle and squamous	1
Spindle and chondroid	1
Chondroid	2
Chondromyxoid	1
Unknown	1
Prior lines of systemic therapy, includes adjuvant and metastatic setting (median)	2 (0, 5)
Prior anti–PD-1 therapy	
Yes	3 (18)
No	14 (82)

Abbreviations: ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; ki67, ki67 nuclear antigen/proliferation index; min/max, minimum/maximum; *N*, number; neg, negative; pos, positive; PR, progesterone receptor.

Two or more responses out of 16 patients were considered evidence that the treatment regimen merits further investigation, provided other data including AEs also appeared satisfactory. This design has 87% power (under an alternative response rate of 30%) with a one-sided alpha of 13% (null response rate assumed to be 5%) in each stratum.

Secondary endpoints were toxicity (per CTCAE version 4), overall survival (OS), and progression-free survival (PFS); PFS was equal for RECIST (12) and iRECIST (14) in this cohort. PFS and OS estimates were calculated utilizing the Kaplan–Meier method (15) and compared using log-rank tests. Confidence intervals (CI) for medians were built using the method of Brookmeyer and Crowley (16); CI for point estimates were calculated employing the log–log transformation. CIs for the primary ORR analysis accounted for the two-stage design and the observed sample size of 17 patients (17). All analyses were performed using R version 4.0.1.

Table 2. Treatment-related adverse events ($N = 17$ patients).

	N (%) of patients		
	Any grade	**Grade 3–4**	**Grade 5**
Any	11 (64.7)	4 (23.5)	1 (5.9)
Serious	4 (23.5)	3 (17.6)	1 (5.9)
Led to discontinuation	1 (5.9)	1 (5.9)[a]	0 (0)
Lead to death	1 (5.9)	0 (0)	1 (5.9)[b]
AE >10% of patients			
AST increased	6 (35.3)	1 (5.9)	0 (0)
Fatigue	5 (29.4)	0 (0)	0 (0)
Adrenal insufficiency	4 (23.5)	1 (5.9)	0 (0)
ALT increased	4 (23.5)	0 (0)	0 (0)
Diarrhea	4 (23.5)	0 (0)	0 (0)
Nausea	3 (17.6)	2 (11.8)	0 (0)
Rash maculopapular	3 (17.6)	1 (5.9)	0 (0)
Abdominal pain	3 (17.6)	0 (0)	0 (0)
Anemia	3 (17.6)	0 (0)	0 (0)
Lymphocyte count decreased	3 (17.6)	0 (0)	0 (0)
Sepsis	2 (11.8)	1 (5.9)	1 (5.9)
Colitis	2 (11.8)	1 (5.9)	0 (0)
Dizziness	2 (11.8)	1 (5.9)	0 (0)
Anorexia	2 (11.8)	0 (0)	0 (0)
Blood bilirubin increased	2 (11.8)	0 (0)	0 (0)
Constipation	2 (11.8)	0 (0)	0 (0)
Headache	2 (11.8)	0 (0)	0 (0)
Hypothyroidism	2 (11.8)	0 (0)	0 (0)
Lipase increased	2 (11.8)	0 (0)	0 (0)
Neck pain	2 (11.8)	0 (0)	0 (0)
Pruritus	2 (11.8)	0 (0)	0 (0)
Vomiting	2 (11.8)	0 (0)	0 (0)
Immune-mediated AE (regardless of frequency)	8 (47.1)	3 (17.6)	0 (0)
AST increased	6 (35.3)	1 (5.9)	0 (0)
Adrenal insufficiency	4 (23.5)	1 (5.9)	0 (0)
ALT increased	4 (23.5)	0 (0)	0 (0)
Diarrhea	4 (23.5)	0 (0)	0 (0)
Rash maculopapular	3 (17.6)	1 (5.9)	0 (0)
Colitis	2 (11.8)	1 (5.9)	0 (0)
Blood bilirubin increased	2 (11.8)	0 (0)	0 (0)
Hypothyroidism	2 (11.8)	0 (0)	0 (0)
Lipase increased	2 (11.8)	0 (0)	0 (0)
Pruritus	2 (11.8)	0 (0)	0 (0)
Hyperthyroidism	1 (5.9)	0 (0)	0 (0)

Abbreviations: ALT, alanine transaminase; AST, aspartate aminotransferase; *N*, number.

[a]Study drug discontinuation per investigator discretion, as patient already had achieved a complete response.

[b]The patient was a 76-year-old female with ECOG PS 2 at the time of enrollment. She had reported urinary frequency around day 14 after first treatment with ipilimumab and nivolumab. She was started on oral antibiotic treatment for urinary tract infection. Presented on day 15 for her next treatment and reported lightheadedness, generalized weakness, poor fluid intake. She developed nausea, emesis, and was noted to be short of breath. Treatment was held and she was referred to the emergency room where she developed pulseless electrical activity and required cardiac resuscitation protocols with return of spontaneous circulation. Laboratory tests showed mild anemia, leukocytosis, normal renal function, elevated troponin (4.79 ng/mL), and transaminitis (AST 339 U/L, ALT 114 U/L, normal 4 days prior). Subsequent CT chest with contrast was negative for pulmonary embolism, and demonstrated grossly stable metastatic disease. EKG post arrest showed anterior ST elevation, cardiac catheterization however revealed normal coronaries and LVEF at 60% to 65%. Hence, non-cardiac causes leading to cardiac arrest were suspected, possibly septic shock. She was treated with broad-spectrum antibiotic therapy, bicarbonates, and vasopressor support. Hemodynamics continued to decline despite multiple vasopressors, and metabolic acidosis worsened despite renal replacement therapy. Due to patient's overall clinical deterioration, her family opted for comfort measures only and patient passed away on day 16 after receiving first dose of both study drugs. Cause of death possibly due to sepsis and possibly related.

Results

Patient characteristics

Overall, 19 patients from 17 National Clinical Trial Network (NCTN) institutions were registered for cohort 36, with 17 patients meeting eligibility criteria and receiving protocol therapy (CONSORT Supplementary Fig. S1). Enrollment was rapid, with 8 eligible patients enrolled January to May 2018 (first stage) followed by a temporary study hold to analyze responses to determine proceeding to the second stage, followed by enrollment of an additional 9 eligible patients from October 2018 to April 2019.

Patient demographics and tumor characteristics are listed in **Table 1**. The median age was 60 years (range, 26–85 years); all patients were female and the majority Caucasian. As expected for MpBC, the majority of tumors were TNBC, high grade, and exhibited high proliferation. Patients had received a median number of two prior lines of systemic therapies, including standard chemotherapies (anthracycline, taxanes, eribulin mesylate, carboplatin, cisplatin, capecitabine), angiogenesis inhibitors, mTOR inhibitors, anti–PD-1 inhibitors, BET inhibitors, HDAC inhibitors, and other investigational agents.

Toxicities

Treatment-related AEs are summarized in **Table 2**. Overall, 11 patients (65%) experienced an AE, with 3 (18%) having a grade 3–4 AE, and 1 grade 5 AE (unknown cause, possibly related, further detail in **Table 2**). Altogether, 47% of participants experienced an irAE; the most common were liver function test (LFT) abnormalities, adrenal insufficiency, and rash.

Outcomes

Efficacy results are shown in **Table 3**. Of 17 enrolled and eligible patients, all of whom had measurable disease, three patients had confirmed objective responses by RECIST 1.1, resulting in an ORR of 18% (95% CI, 6%–40%). Notably, all three responses have been durable and have been ongoing at 28+, 33+, and 34+ months, respectively, and are therefore considered exceptional (**Fig. 1**). These responses were observed in spindle cell MpBC ($n = 2$) and chondromyxoid MpBC ($n = 1$). Nonresponders had poor outcomes; stable disease (SD) was seen in 18%, none lasting > 6 months. Median PFS and OS were 2 and 12 months, respectively (**Fig. 1**). Median follow-up among patients who are alive is 33 months. Cut-off date of data follow-up is February 4, 2021. Three patients had received prior anti–PD-1 therapy, none of whom had a tumor response.

Table 3. Best response summary by RECIST 1.1 ($N = 17$ patients).

	Patient number (%) or time
CR	1 (6%)
PR	2 (12%)
ORR (CR + PR)	3 (18%)
SD (all <6 months)	3 (18%)
PD[a]	11 (65%)
Duration of response	28+, 33+, 34+ months, all ongoing
PFS at 6 months	18% (6%, 49%)
Median PFS and OS	2 and 12 months

Abbreviations: CR, complete response; PD, progressive disease; PR, partial response; SD, stable disease.
[a]Includes patients who progressed or died before first on-study assessment.

Exceptional responders are described in more detail in Supplementary Table S1. All three responders had chemotherapy-refractory disease with significant tumor burden. The tumors' baseline target lesion sum ranged from 6.1 to 11.3 cm and cancers had recurred within one year of a taxane and/or anthracycline-containing regimen, or progressed on it. None had received prior immunotherapy. Reassuringly, responses persisted despite stopping ipilimumab in one patient and ipilimumab + nivolumab in another patient (**Fig. 1**). Of note, all three responders developed significant irAEs with adrenal insufficiency induced in all three.

Genomic alterations and PD-L1 expression

Prespecified trial correlative studies have been delayed due to COVID-19 pandemic work restrictions. Molecular and immunohistochemistry (IHC) tumor characterization done as part of routine medical care is shown for all three responding patients in Supplementary Table S1. Tumors had low tumor mutational burden (TMB), were microsatellite stable, and two of three had negative or low PD-L1 expression/TILs.

Discussion

To our knowledge, our study represents the first prospective trial of immunotherapy in MpBC, a rare subset of TNBC. This cohort of the DART trial met its primary endpoint: ipilimumab plus nivolumab was clinically active in advanced MpBC, with responses observed in 3 of 17 patients (ORR 18%). Importantly, all responses were durable (28+, 33+, and 34+ months), which is rarely observed in MpBC.

Advanced MpBC has a poor prognosis with median OS less than one year despite chemotherapy (18); therefore, new treatment strategies are urgently needed. MpBCs harbor a wide variety of genomic alterations, the most frequent being in the *TP53* and *PIK3CA* genes (9). Therapies targeting the PI3K/AKT/mTOR pathway and antiangiogenesis agents have shown objective responses in a subset of patients (18–21). The limited treatment options and poor prognosis of MpBC along with the high tumoral PD-L1 expression and TIL presence in some patients (8) provided the rationale to study immunotherapies in this subtype.

Objective responses to dual immunotherapy were seen in three patients, two with spindle cell and one with chondromyxoid histology. All three women had chemotherapy-refractory disease with significant tumor burden (range of baseline target lesion sum 6.1–11.3 cm). These responses are remarkable for several reasons. First, they were durable with all responses ongoing beyond 2 years, which is in stark contrast to the short-lived responses observed with chemotherapy (18) as well as with anti–PD-1 blockade in MpBC (10). Furthermore, three of the patients in our study (all nonresponders) who had received prior therapy with anti–PD-1 (combined with platinum chemotherapy, targeted agents, or a STING agonist) rapidly progressed on that therapy, all within 2 months. Second, responses were observed in patients with highly chemotherapy-refractory disease, as evidenced by disease recurrence within one year of a taxane and/or anthracycline combination regimen or progression on it, known to be an independent poor prognostic factor for TNBC (22–24). Third, responses were observed even in tumors with negative or low PD-L1 expression and low TIL. This may suggest a contribution of the anti-CTLA blockade to the efficacy of the regimen studied, as responses to anti–PD-1/anti–PD-L1 therapeutics are enriched in PD-L1–positive tumors in metastatic TNBC (23, 25). Fourth, all three responders developed significant irAEs including panhypopituitarism and adrenal insufficiency. While irAE toxicity has been observed across immune checkpoint

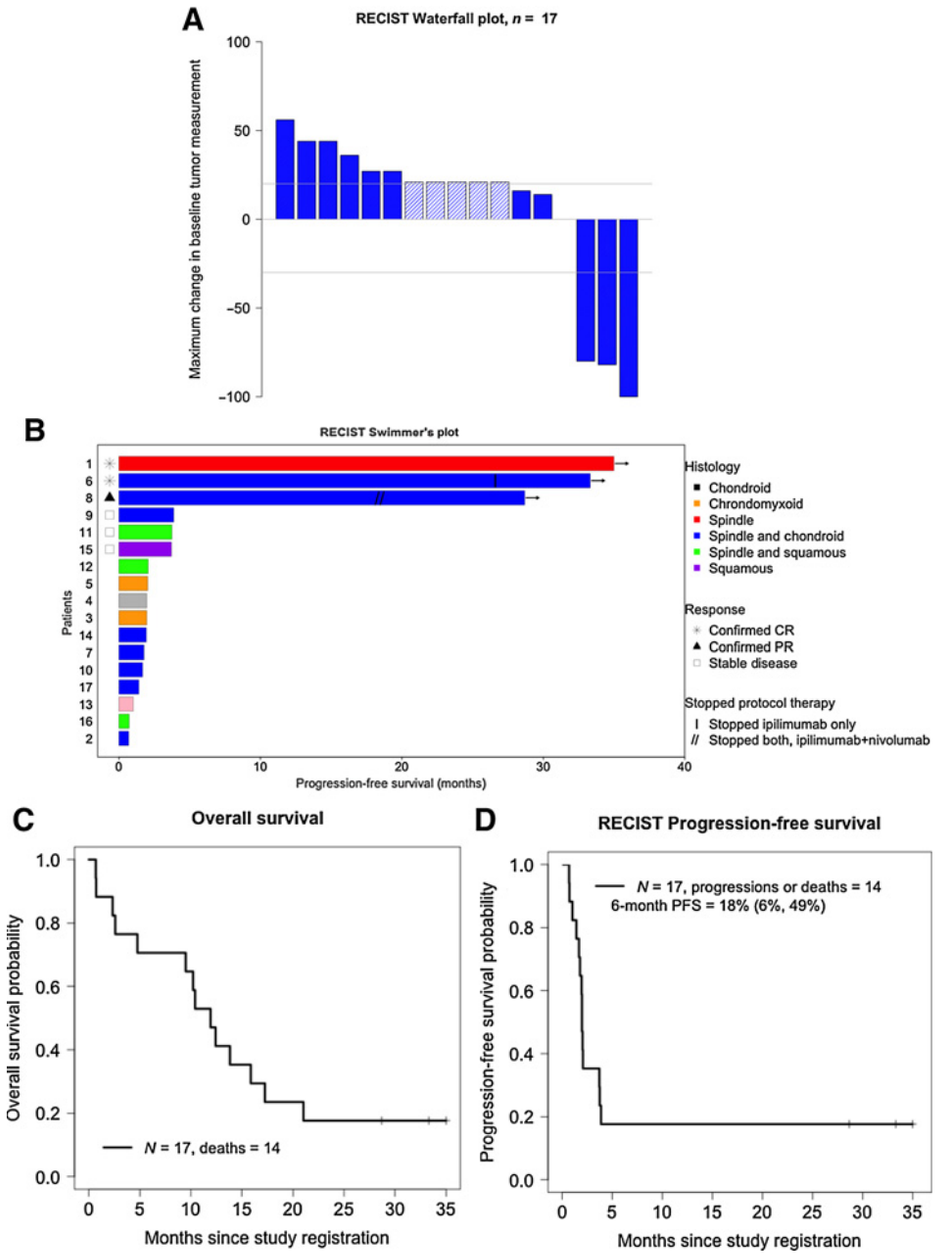

Figure 1.

Outcome of patients with metaplastic breast cancer treated with ipilimumab and nivolumab (RECIST 1.1). **A,** Waterfall plot. Horizontal lines mark RECIST progression (+20%) and PR (−30%). Crosshatch indicates patient failed therapy and does not have tumor measurements available due progression [due to new lesions at first assessment ($n = 3$), death before assessment ($n = 1$), or withdrew consent for follow-up when entered hospice before first assessment ($n = 1$)]. One patient had 0% change in RECIST measurements and therefore appears as a gap in the waterfall. **B,** Swimmer's plot. By MpBC histology. **C** and **D,** OS and PFS Kaplan–Meier curve.

inhibitor regimens and has been shown to correlate with antitumor responses in some studies (26), the duration of treatment is generally longer in responders, which could be a confounder due to longer exposure to therapy. Adrenal insufficiency, for instance, was diagnosed around cycle 3 to 4 at approximately 4 months in the responders, at which time most of the nonresponder patients had come off study for progression. Fifth, our responders had low TMB. TMB can be strongly correlated with responsiveness to checkpoint blockade, with only approximately 5% of patients responding to anti–PD-1/PD-L1 agents when TMB is low (≤5 mutations/mb); however, our prior studies suggest that responses to anti–CTLA-4/anti–PD-1/PD-L1 combinations, as given in the current study, are independent of TMB (27).

While the contribution of CTLA-4 blockade to the efficacy of the anti–CTLA-4/anti–PD-1 combination and in particular the duration of responses remains unknown in our trial, we would like to reference efficacy data of anti–PD-1/PD-L1 therapy in MpBC as well as TNBC in general. Data from a recently published case series of MpBC, which included 4 patients from an investigator-initiated trial of anti–PD-1 therapy (with capecitabine or paclitaxel) as well as one patient treated off-label with pembrolizumab and bicalutamide), demonstrated objective responses in 3 patients; however, PFS was only 5.3, 5.7, and 8.0 months (28). In another investigator-initiated trial of anti–PD-1 therapy with nab-paclitaxel, 1 of 2 patients with MpBC experienced an objective response; however, PFS was only 6.7 months (Adams et al, NCT02752685, unpublished data). In phase III trials of chemoimmunotherapy for metastatic (general) TNBC, durable responses were observed; however, the percentage of patients with ongoing responses years out is very small. For instance in our final analysis

of Impassion130, among patients alive at 3 years, only 16 patients had not experienced progression of disease, representing 1.77% of the 902 patients enrolled and 2.66% of patients treated with the chemo-immunotherapy combination (12/451; ref. 29).

As discussed above, responses to chemo- plus anti–PD-1 therapy observed in MpBC are typically not durable (all ≤9 months) and combinatorial immunotherapies may be required to achieve durable responses. However, it is important to note the addition of anti–CTLA-4 to anti–PD-1/PD-L1 regimens has been associated with greater toxicity and higher mortality rates (30, 31). A meta-analysis of 112 trials involving 19,217 patients showed toxicity-related fatality rates of 0.36% (anti–PD-1), 0.38% (anti–PD-L1), 1.08% (anti–CTLA-4), and 1.23% (anti–PD-1/PD-L1 plus anti–CTLA-4; ref. 30).

The side effect profile of the anti–CTLA-4/anti–PD-1 combination in our study was consistent with published combination studies (31, 32) and that observed in other cohorts of DART. No unexpected toxicities were observed, however, the fatal event and several potentially life-threatening AEs such as adrenal insufficiency highlight the importance of a thorough risk–benefit discussion with patients and their education about possible side effects before treatment start as well as careful monitoring of patients on treatment by oncologists at centers with immunotherapy experience. Furthermore, it is essential to raise awareness among emergency department physicians, critical care providers, and other specialists, especially as serious toxicities can have unusual clinical presentations and grade 5 toxicities can occur very early in the treatment course, as shown for ipilimumab combination therapies with a median 14.5 days (30). Subsequent studies should therefore carefully consider these risks and possibly modify dosing, as AEs have been shown to occur more often at higher doses of ipilimumab (30), or evaluate newer anti–CTLA-4 formulations such as probodies to widen the therapeutic window (33).

To better understand the molecular basis for responses, correlative samples were collected on study and will be analyzed as per pre-specified plan at Cancer Immune Monitoring and Analysis Centers (CIMAC) sites. Some patients had tumor NGS performed for clinical purposes outside of the DART trial and results were available as part of the medical record. Based on these local data, tumors of responders with NGS available showed low TMB and absence of microsatellite instability (MSI), which is consistent with our published large dataset of 192 MpBC demonstrating a low TMB across these tumors (median 2.7 mutations/Mb) along with microsatellite stability (0/192 MpBC were MSI high; ref. 9).

The strengths of our study include the enrollment of patients at both academic and community centers as well as support from the NCI and SWOG. Furthermore, DART served an unmet need with rare tumors and demonstrated that it was feasible to rapidly accrue even very rare tumors (11), especially because SWOG was able to mobilize >800 sites for the DART study. Limitations of the study include a relatively small sample size within a single arm design per disease cohort, and the lack of a randomized comparison with standard-of-care therapies. Central pathology review was not mandated, and therefore we relied on local site assessments that may be suboptimal for rare occurring cancers; however, review of all pathology reports was conducted by the study chair, confirming the presence of metaplastic components in specimens. Furthermore, for the three responders, digital pathology images were reviewed for assessment of TILs.

In conclusion, among 17 patients treated with nivolumab and ipilimumab, three exceptional responses were observed in chemotherapy-refractory, metastatic MpBC, all ongoing at 28+, 33+, and 34+ months. Further investigation of this combination is warranted. Of special interest, in this study, responses were dichotomized into the 18% that did remarkably well versus the others that had no significant benefit from ipilimumab and nivolumab. Such a dichotomization may suggest the presence of a unique biomarker for response in these patients and should be taken into consideration in the design of a future trial, possibly with an adaptive design. Interestingly, the exceptional responders included patients whose tumors had low TMB, low TIL, and no PD-L1 expression, indicating that the mechanism of response requires further in-depth interrogation, which is planned via collaboration with CIMAC sites.

Authors' Disclosures

S. Adams reports other support from BMS during the conduct of the study. M. Othus reports grants from National Institutes of Health during the conduct of the study, as well as personal fees from Merck, Biosight, Celgene, Glycomimetics, and Daiichi Sankyo outside the submitted work. S.P. Patel reports grants and personal fees from BMS during the conduct of the study, as well as grants and personal fees from Amgen, AstraZeneca, Bristol Myers Squibb, Certis, Eli Lilly, Genentech, Illumina, Merck, Pfizer, Rakuten, and Tempus outside the submitted work. K.D. Miller reports other support from Merck, Roche/Genentech, and AstraZeneca, as well as grants from Pfizer and Astex outside the submitted work. R. Chugh reports grants and personal fees from Epizyme, Inc.; personal fees from Ipsen and Deciphera; grants from Qilu Puget Sound, AADi, Novartis, Medivation, Advenchen, Plexxikonn, Springworks, Ayala, and Mundipharma; grants and non-financial support from GlaxoSmithKline; and non-financial support from Janssen and AstraZeneca outside the submitted work. A.P. O'Dea reports personal fees from Puma Biotechnology, Pfizer, Daiichi Sankyo, AstraZeneca, and Novartis outside the submitted work. H.R. Mirshahidi reports other support from Merck and Takeda outside the submitted work. J. Rodon Ahnert reports grants and personal fees from Novartis, Spectrum Pharmaceuticals Inc., and Pfizer; personal fees from Eli Lilly, Orion Pharmaceuticals, Servier Pharmaceuticals, Peptomyc, Roche Pharmaceuticals, Ellipses Pharma, Certera, NovellusDX, and IONCTURA SA; personal fees and other support from Molecular Partners, Merck Sharp & Dohme and Kelun Pharmaceuticals/Klus Pharma; grants, personal fees, and other support from Bayer; grants from Ipsen, Blueprint Medicines, Tocagen, Symphogen, BioAlta, GenMab, CytomX, Kelun-Biotech, and Takeda-Miillenium; grants and other support from GlaxoSmithKline; and other support from ESMO, Department of Defense, Louisiana State University, Huntsman Cancer Institute, Cancer Core Europe, Karolinska Cancer Institute, King Abdullah International Medical Research Center, WIN Consortium, Janssen, European Journal of Cancer, VHIO/Ministero De Empleo Y Seguridad Social, Chinese University of Hong Kong, SOLTI, and Elsevier outside the submitted work. R.M. Connolly reports grants from Pfizer, Genentech, Novartis, Puma Biotechnology, Merck, and Macrogenics outside the submitted work. R.J. Gray reports grants from National Cancer Institute during the conduct of the study. C.D. Blanke reports grants from NCI during the conduct of the study. Y.K. Chae reports grants and personal fees from BMS during the conduct of the study. R. Kurzrock reports grants, personal fees, non-financial support, and other support from Biological Dynamics, Boehringer Ingelheim, Debiopharm, Foundation Medicine, Genentech, Grifols, Guardant, Incyte, Konica Minolta, Medimmune, Merck Serono, Omniseq, Pfizer, Sequenom, Takeda, and TopAlliance; is a consultant or advisory board member and/or has received speaker fees from Actuate Therapeutics, AstraZeneca, Bicara Therapeutics, Biological Dynamics, Eisai, EOM Pharmaceuticals, Iylon, Merck, NeoGenomics, Neomed, Pfizer, Prosperdtx, Roche, TD2/Volastra, Turning Point Therapeutics, and X-Biotech; has an equity interest in CureMatch Inc., CureMetrix, and IDbyDNA; serves on the board of CureMatch and CureMetrix; and is a co-founder of CureMatch outside the submitted work. No disclosures were reported by the other authors.

Authors' Contributions

S. Adams: Conceptualization, resources, data curation, formal analysis, supervision, investigation, writing–original draft, writing–review and editing. **M. Othus:** Conceptualization, resources, data curation, software, formal analysis, supervision, funding acquisition, investigation, visualization, methodology, writing–original draft, project administration, writing–review and editing. **S.P. Patel:** Conceptualization, resources, supervision, funding acquisition, investigation, methodology, project administration, writing–review and editing. **K.D. Miller:** Conceptualization, writing–review and editing. **R. Chugh:** Investigation. **S.M. Schuetze:** Investigation. **M.D. Chamberlin:** Data curation, investigation. **B.J. Haley:** Data curation, investigation. **A.V. Storniolo:** Data curation, investigation. **M.P. Reddy:** Investigation. **S.A. Anderson:** Investigation. **C.T. Zimmerman:** Investigation. **A.P. O'Dea:**

Investigation. **H.R. Mirshahidi:** Investigation, writing–review and editing. **J. Rodon Ahnert:** Conceptualization, resources, investigation, writing–review and editing. **F.J. Brescia:** Conceptualization, resources, investigation, writing–review and editing. **O. Hahn:** Conceptualization, resources, investigation, writing–review and editing. **J.M. Raymond:** Investigation. **D.D. Biggs:** Conceptualization, resources, formal analysis, supervision, investigation, methodology, writing–review and editing. **R.M. Connolly:** Conceptualization, resources, formal analysis, supervision, investigation, methodology, writing–review and editing. **E. Sharon:** Conceptualization, resources, formal analysis, funding acquisition, methodology, project administration, writing–review and editing. **L.A. Korde:** Conceptualization, resources, supervision, funding acquisition, investigation, methodology, project administration, writing–review and editing. **R.J. Gray:** Conceptualization, resources, formal analysis, supervision, funding acquisition, investigation, methodology, project administration, writing–review and editing. **E. Mayerson:** Conceptualization, resources, formal analysis, supervision, funding acquisition, investigation, methodology, project administration, writing–review and editing. **M. Plets:** Conceptualization, resources, formal analysis, funding acquisition, investigation, methodology, project administration. **C.D. Blanke:** Conceptualization, resources, supervision, funding acquisition, investigation, methodology, project administration, writing–review and editing. **Y.K. Chae:** Conceptualization, resources, supervision, funding acquisition, investigation, methodology, project administration, writing–review and editing. **R. Kurzrock:** Conceptualization, resources, supervision, funding acquisition, investigation, methodology, project administration, writing–review and editing.

Acknowledgments

Research reported in this publication was supported by the National Cancer Institute of the National Institutes of Health under grant award numbers U10CA180888, U010CA180819, U10CA180820, U10CA180821, U10180868, U10CA180794, and UG1CA233196, and in part by Bristol-Myers Squibb Company. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health or the Bristol-Meyers Squibb Company.

We would like to thank the patients who participated in this trial and their families, as well as the study teams of participating institutions. We also thank Dr. Roberto Salgado for scoring the TILs for the responders.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received June 14, 2021; revised August 12, 2021; accepted October 25, 2021; published first October 29, 2021.

References

1. Weigelt B, Eberle C, Cowell CF, Ng CK, Reis-Filho JS. Metaplastic breast carcinoma: more than a special type. Nat Rev Cancer 2014;14:147–8.
2. Lakhani SR, Ellis IO, Schnitt SJ, Tan PH, van de Vijver MJ. Metaplastic carcinoma. WHO classification of tumours of the breast. 4th edition. Lyon, France: International Agency for Research on Cancer (IARC); 2012:48–52.
3. Avigdor BE, Beierl K, Gocke CD, Zabransky DJ, Cravero K, Kyker-Snowman K, et al. Whole-exome sequencing of metaplastic breast carcinoma indicates monoclonality with associated ductal carcinoma component. Clin Cancer Res 2017;23:4875–84.
4. Wong W, Brogi E, Reis-Filho JS, Plitas G, Robson M, Norton L, et al. Poor response to neoadjuvant chemotherapy in metaplastic breast carcinoma. NPJ Breast Cancer 2021;7:96.
5. Aydiner A, Sen F, Tambas M, Ciftci R, Eralp Y, Saip P, et al. Metaplastic breast carcinoma versus triple-negative breast cancer: survival and response to treatment. Medicine 2015;94:e2341.
6. Hennessy BT, Gonzalez-Angulo AM, Stemke-Hale K, Gilcrease MZ, Krishnamurthy S, Lee JS, et al. Characterization of a naturally occurring breast cancer subset enriched in epithelial-to-mesenchymal transition and stem cell characteristics. Cancer Res 2009;69:4116–24.
7. Reddy TP, Rosato RR, Li X, Moulder S, Piwnica-Worms H, Chang JC. A comprehensive overview of metaplastic breast cancer: clinical features and molecular aberrations. Breast Cancer Res 2020;22:121.
8. Joneja U, Vranic S, Swensen J, Feldman R, Chen W, Kimbrough J, Xiao N, Reddy S, Palazzo J, Gatalica Z. Comprehensive profiling of metaplastic breast carcinomas reveals frequent overexpression of programmed death-ligand 1. J Clin Pathol 2017;70:255–9.
9. Tray N, Taff J, Singh B, Suh J, Ngo N, Kwa M, et al. Metaplastic breast cancers: Genomic profiling, mutational burden and tumor-infiltrating lymphocytes. Breast 2019;44:29–32.
10. Adams S. Dramatic response of metaplastic breast cancer to chemo-immunotherapy. NPJ Breast Cancer 2017;3:8.
11. Patel SP, Othus M, Chae YK, Giles FJ, Hansel DE, Singh PP, et al. A phase II basket trial of dual anti-CTLA-4 and anti-PD-1 blockade in rare tumors (DART SWOG 1609) in patients with non-pancreatic neuroendocrine tumors. Clin Cancer Res 2020;26:2290–6.
12. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer 2009;45:228–47.
13. Wagner MJ, Othus M, Patel SP, Ryan C, Sangal A, Powers B, et al. Multicenter phase II trial (SWOG S1609, cohort 51) of ipilimumab and nivolumab in metastatic or unresectable angiosarcoma: a substudy of dual anti-CTLA-4 and anti-PD-1 blockade in rare tumors (DART). J Immunother Cancer 2021;9:e002990.
14. Seymour L, Bogaerts J, Perrone A, Ford R, Schwartz LH, Mandrekar S, et al. iRECIST: guidelines for response criteria for use in trials testing immunotherapeutics. Lancet Oncol 2017;18:e143–52.
15. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. J Am Statist Assoc 1958;53:457–81.
16. Brookmeyer R, Crowley J. A confidence interval for the median survival time. Biometrics 1982;38:29–41.
17. Koyama T, Chen H. Proper inference from Simon's two-stage designs. Stat Med 2008;27:3145–54.
18. Tray N, Taff J, Adams S. Therapeutic landscape of metaplastic breast cancer. Cancer Treat Rev 2019;79:101888.
19. Moulder S, Helgason T, Janku F, Wheler J, Moroney J, Booser D, et al. Inhibition of the phosphoinositide 3-kinase pathway for the treatment of patients with metastatic metaplastic breast cancer. Ann Oncol 2015;26:1346–52.
20. Moulder S, Moroney J, Helgason T, Wheler J, Booser D, Albarracin C, et al. Responses to liposomal doxorubicin, bevacizumab, and temsirolimus in metaplastic carcinoma of the breast: biologic rationale and implications for stem-cell research in breast cancer. J Clin Oncol 2011;29:e572–5.
21. Moroney J, Fu S, Moulder S, Falchook G, Helgason T, Levenback C, et al. Phase I study of the antiangiogenic antibody bevacizumab and the mTOR/hypoxia-inducible factor inhibitor temsirolimus combined with liposomal doxorubicin: tolerance and biological activity. Clin Cancer Res 2012;18:5796–805.
22. Adams S, Loi S, Toppmeyer D, Cescon DW, De Laurentiis M, Nanda R, et al. Pembrolizumab monotherapy for previously untreated, PD-L1-positive, metastatic triple-negative breast cancer: cohort B of the phase II KEYNOTE-086 study. Ann Oncol 2019;30:405–11.
23. Cortes J, Cescon DW, Rugo HS, Nowecki Z, Im SA, Yusof MM, et al. Pembrolizumab plus chemotherapy versus placebo plus chemotherapy for previously untreated locally recurrent inoperable or metastatic triple-negative breast cancer (KEYNOTE-355): a randomised, placebo-controlled, double-blind, phase 3 clinical trial. Lancet 2020;396:1817–28.
24. Kassam F, Enright K, Dent R, Dranitsaris G, Myers J, Flynn C, et al. Survival outcomes for patients with metastatic triple-negative breast cancer: implications for clinical practice and trial design. Clin Breast Cancer 2009;9:29–33.
25. Schmid P, Adams S, Rugo HS, Schneeweiss A, Barrios CH, Iwata H, et al. Atezolizumab and nab-paclitaxel in advanced triple-negative breast cancer. N Engl J Med 2018;379:2108–21.
26. Xing P, Zhang F, Wang G, Xu Y, Li C, Wang S, et al. Incidence rates of immune-related adverse events and their correlation with response in advanced

solid tumours treated with NIVO or NIVO+IPI: a systematic review and meta-analysis. J Immunother Cancer 2019;7:341.
27. Goodman AM, Kato S, Bazhenova L, Patel SP, Frampton GM, Miller V, et al. Tumor mutational burden as an independent predictor of response to immunotherapy in diverse cancers. Mol Cancer Ther 2017; 16:2598–608.
28. Kim I, Rajamanickam V, Bernard B, Chun B, Wu Y, Martel M, et al. A case series of metastatic metaplastic breast carcinoma treated with anti-PD-1 therapy. Front Oncol 2021;11:635237.
29. Emens LA, Adams S, Barrios CH, Dieras V, Iwata H, Loi S, et al. First-line atezolizumab plus nab-paclitaxel for unresectable, locally advanced, or metastatic triple-negative breast cancer: IMpassion130 final overall survival analysis. Ann Oncol 2021;32:983–93.
30. Wang DY, Salem JE, Cohen JV, Chandra S, Menzer C, Ye F, et al. Fatal toxic effects associated with immune checkpoint inhibitors: a systematic review and meta-analysis. JAMA Oncol 2018;4:1721–8.
31. D'Abreo N, Adams S. Immune-checkpoint inhibition for metastatic triple-negative breast cancer: safety first? Nat Rev Clin Oncol 2019; 16:399–400.
32. Larkin J, Chiarion-Sileni V, Gonzalez R, Grob JJ, Cowey CL, Lao CD, et al. Combined nivolumab and ipilimumab or monotherapy in untreated melanoma. N Engl J Med 2015;373:23–34.
33. Autio KA, Boni V, Humphrey RW, Naing A. Probody therapeutics: an emerging class of therapies designed to enhance on-target effects with reduced off-tumor toxicity for use in immuno-oncology. Clin Cancer Res 2020;26:984–9.