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Association of Circulating Tumor DNA and Circulating Tumor Cells After Neoadjuvant Chemotherapy With Disease Recurrence in Patients With Triple-Negative Breast Cancer

Preplanned Secondary Analysis of the BRE12-158 Randomized Clinical Trial

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IMPORTANCE A significant proportion of patients with early-stage triple-negative breast cancer (TNBC) are treated with neoadjuvant chemotherapy. Sequencing of circulating tumor DNA (ctDNA) after surgery, along with enumeration of circulating tumor cells (CTCs), may be used to detect minimal residual disease and assess which patients may experience disease recurrence.

OBJECTIVE To determine whether the presence of ctDNA and CTCs after neoadjuvant chemotherapy in patients with early-stage TNBC is independently associated with recurrence and clinical outcomes.

DESIGN, SETTING, AND PARTICIPANTS A preplanned secondary analysis was conducted from March 26, 2014, to December 18, 2018, using data from 196 female patients in BRE12-158, a phase 2 multicenter randomized clinical trial that randomized patients with early-stage TNBC who had residual disease after neoadjuvant chemotherapy to receive postneoadjuvant genomically directed therapy vs treatment of physician choice. Patients had blood samples collected for ctDNA and CTCs at time of treatment assignment; ctDNA analysis with survival was performed for 142 patients, and CTC analysis with survival was performed for 123 patients. Median clinical follow-up was 17.2 months (range, 0.3-58.3 months).

INTERVENTIONS Circulating tumor DNA was sequenced using the FoundationACT or FoundationOneLiquid Assay, and CTCs were enumerated using an epithelial cell adhesion molecule–based, positive-selection microfluidic device.

MAIN OUTCOMES AND MEASURES Primary outcomes were distant disease–free survival (DDFS), disease-free survival (DFS), and overall survival (OS).

RESULTS Among 196 female patients (mean [SD] age, 49.6 [11.1] years), detection of ctDNA was significantly associated with inferior DDFS (median DDFS, 32.5 months vs not reached; hazard ratio [HR], 2.99; 95% CI, 1.38-6.48; $P = .006$). At 24 months, DDFS probability was 56% for ctDNA-positive patients compared with 81% for ctDNA-negative patients. Detection of ctDNA was similarly associated with inferior DFS (HR, 2.67; 95% CI, 1.28-5.57; $P = .009$) and inferior OS (HR, 4.16; 95% CI,1.66-10.42; $P = .002$). The combination of ctDNA and CTCs provided additional information for increased sensitivity and discriminatory capacity. Patients who were ctDNA positive and CTC positive had significantly inferior DDFS compared with those who were ctDNA negative and CTC negative (median DDFS, 32.5 months vs not reached; HR, 5.29; 95% CI, 1.50-18.62; $P = .009$). At 24 months, DDFS probability was 52% for patients who were ctDNA positive and CTC positive compared with 89% for those who were ctDNA negative and CTC negative. Similar trends were observed for DFS (HR, 3.15; 95% CI, 1.07-9.27; $P = .04$) and OS (HR, 8.60; 95% CI, 1.78-41.47; $P = .007$).

CONCLUSIONS AND RELEVANCE In this preplanned secondary analysis of a randomized clinical trial, detection of ctDNA and CTCs in patients with early-stage TNBC after neoadjuvant chemotherapy was independently associated with disease recurrence, which represents an important stratification factor for future postneoadjuvant trials.

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Supplemental content

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A large proportion of patients with triple-negative breast cancer (TNBC) are treated with neoadjuvant chemotherapy. Approximately one-third of patients will achieve a pathologic complete response with neoadjuvant chemotherapy and have favorable outcomes. In contrast, two-thirds of patients will have residual disease and are at high risk of relapse.[1] Methods that can detect the presence of minimal residual disease (MRD) in the circulation after surgery may be used to determine in which patients disease will recur.

An established method for detection of MRD is the analysis of circulating tumor DNA (ctDNA). Because somatic mutations provide intrinsic specificity for nucleic acid material derived from tumor tissue, the presence of ctDNA implies the presence of disease. Our group and others have demonstrated that ctDNA detected after neoadjuvant chemotherapy and surgery in the plasma of patients with TNBC is associated with rapid relapse.[2,3] Another commonly used analyte from liquid biopsies are circulating tumor cells (CTCs).[4] These cells are frequently detected in both early-stage and late-stage breast cancers; enumeration of these cells is associated with prognosis in breast cancer.[5-8] Under certain circumstances, CTCs can be isolated from the circulation in the absence of detectable ctDNA. This occurs primarily when the index mutations are not covered by the ctDNA assay or owing to very low concentrations or shedding of ctDNA.[9] Herein, using ctDNA and CTCs prospectively collected after neoadjuvant chemotherapy and surgery from patients with TNBC, we analyzed the association of liquid biopsy–based MRD with clinical outcomes.

Key Points

Question Is the presence of circulating tumor DNA and circulating tumor cells after surgery associated with inferior outcomes for patients with early-stage triple-negative breast cancer?

Findings This large preplanned secondary analysis of 196 female patients from a recently completed randomized clinical trial found that the presence of circulating tumor DNA and circulating tumor cells after neoadjuvant chemotherapy in patients with early-stage triple-negative breast cancer was associated with significantly inferior distant disease–free survival, disease-free survival, and overall survival.

Meaning Detection of circulating tumor DNA and circulating tumor cells after neoadjuvant chemotherapy in patients with early-stage triple-negative breast cancer is independently associated with disease recurrence, above and beyond standard clinical parameters, and represents an important novel stratification factor for future postneoadjuvant trials.

Methods

The BRE12-158 study was a phase 2 randomized clinical trial of genomically directed therapy after preoperative chemotherapy for patients with TNBC (NCT02101385) (trial protocol in Supplement 1; eFigure 1A in Supplement 2). This multicenter trial enrolled patients with TNBC treated with neoadjuvant chemotherapy who had residual disease at the time of surgery. Blood samples for the possible detection of ctDNA and CTCs were obtained either prior to treatment on day 1 of chemotherapy treatment cycle 1 for arm A or at the first routine visit for arm B. A CONSORT diagram of patient selection is outlined in eFigure 1B in Supplement 2. Patient characteristics are detailed in eTable 1 in Supplement 2. All patients provided written informed consent, and the study was approved by the institutional review boards of Indiana University Melvin and Bren Simon Cancer Center, Froedtert & The Medical College of Wisconsin, Georgetown University, University of Chicago, University of Alabama at Birmingham, University of Florida, Virginia Oncology Associates, Meritus Center for Clinical Research, Community Regional Cancer Care, Memorial Cancer Institute, Erlanger Health System, University of Miami, University of Cincinnati Cancer Institute, Washington University School of Medicine, IU Health Goshen Center for Cancer Care, Nebraska Methodist Hospital, Winship Cancer Institute of Emory University, Joe Arrington Cancer Research and Treatment Center, Aurora Health Care, PinnacleHealth Cancer Center, Fort Wayne Medical Oncology and Hematology, IU Health Arnett, Mercy Clinic Oklahoma Communities, Tufts Medical Center, and Community Hospital of Anderson and Madison County Inc. Sequencing of ctDNA was performed using the FoundationACT or FoundationOne Liquid assays (Foundation Medicine Inc), as previously described.[10] Circulating tumor cells were detected using an epithelial cell adhesion molecule-based positive-selection microfluidic device.[11-14] All survival analyses are the product of multivariate analyses. Detailed methods are provided in the eMethods in Supplement 2.

Results

Association of ctDNA With Clinical Outcomes

Circulating tumor DNA samples were sequenced, and mutations were filtered to identify those that had the highest likelihood to be somatic (eFigure 2 in Supplement 2). Circulating tumor DNA positivity was consistent across both groups, with 65% of patients (37 of 57) positive for ctDNA in arm A and 62% of patients (53 of 85) positive for ctDNA in arm B. Median clinical follow-up was 17.2 months (range, 0.1-58.3 months). Detection of ctDNA was significantly associated with an inferior DDFS (median DDFS, 32.5 months vs not reached; hazard ratio [HR], 2.99; 95% CI, 1.38-6.48; $P = .006$) (**Figure 1**A). At 24 months, the DDFS probability was 56% for ctDNA-positive patients compared with 81% for ctDNA-negative patients. Similarly, detection of ctDNA was significantly associated with an inferior DFS (median DFS, 22.8 months vs not reached; HR, 2.67; 95% CI, 1.28-5.57; $P = .009$) (Figure 1B). At 24 months, the DFS probability was 50% for ctDNA-positive patients compared with 76% for ctDNA-negative patients. Last, detection of ctDNA was significantly associated with an inferior OS (median OS, not reached vs not reached; HR, 4.16; 95% CI, 1.66-10.42; $P = .002$) (Figure 1C). At 24 months, the OS probability was 57% for ctDNA-positive patients compared with 80% for ctDNA-negative patients.

Association of the Combination of CTCs and ctDNA With Clinical Outcomes

Circulating tumor cell positivity was detected in 43% of patients (21 of 49) in group A and 39% of patients (29 of 74) in group B. Although patients who were CTC positive had inferior outcomes, results did not reach statistical significance (eFigure 3 in Supplement 2). Increasing CTC count, however, was significantly associated with inferior DDFS (HR, 1.07; 95% CI, 1.01-1.13; P = .02), DFS (HR, 1.11; 95% CI, 1.03-1.19; P = .004), and OS (HR, 1.09; 95% CI, 1.02-1.17; P = .01), suggesting that the quantitative burden of CTCs is associated with outcomes. Circulating tumor cells may provide additional information about the presence of MRD. Specifically, for the 112 patients for whom both ctDNA and CTC results were available, we did not find a significant association between CTC positivity (defined as ≥1 CTC detected) and ctDNA positivity (P = .19). A proportion of patients were positive for 1 marker and not the other, such that the sensitivity to detect recurrences went from 79% (23 of 29) with ctDNA alone and 62% (18 of 29) with CTC alone to 90% (26 of 29) when combined (eFigure 4 in Supplement 2).

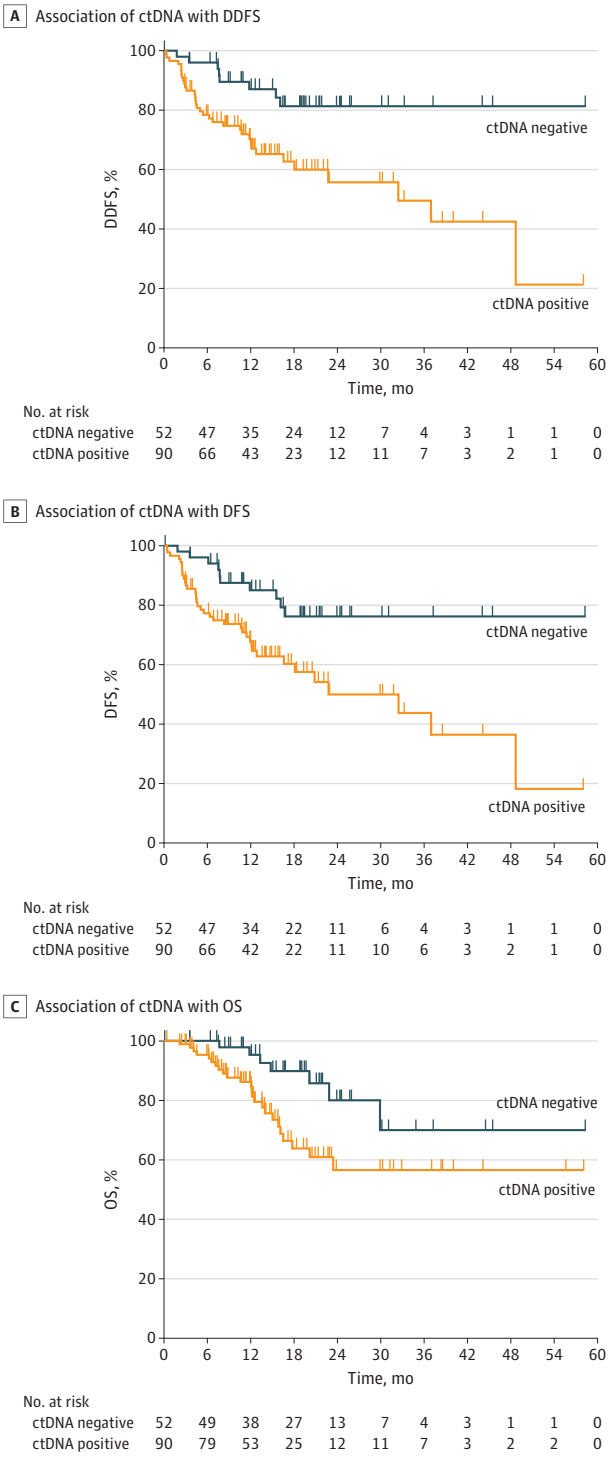
We combined the data on ctDNA and CTCs to compare the DDFS curves for the following 4 groups of patients (1) ctDNA positive and CTC positive, (2) ctDNA positive and CTC negative, (3) ctDNA negative and CTC positive, and (4) ctDNA negative and CTC negative. The DDFS curves demonstrated a stepwise gradation in which patients who were positive for both ctDNA and CTCs had inferior DDFS compared with those who were positive for ctDNA alone or CTC alone, and patients who were negative for both ctDNA and CTCs had the best outcomes (**Figure 2**A). Patients who were ctDNA positive and CTC positive had a significantly inferior DDFS compared with those who were ctDNA negative and CTC negative (median DDFS, 32.5 months vs not reached; HR, 5.29; 95% CI, 1.50-18.62; P = .009) (Figure 2A). At 24 months, the DDFS probability was 52% for patients who were ctDNA positive and CTC positive compared with 89% for those who were ctDNA negative and CTC negative. We observed similar trends when analyzing DFS (median DFS, 20.8 months vs not reached; HR, 3.15; 95% CI, 1.07-9.27; P = .04) (Figure 2B) and OS (median OS, not reached vs not reached; HR, 8.60; 95% CI, 1.78-41.47; P = .007) (Figure 2C) among patients who were ctDNA positive and CTC positive compared with those who were ctDNA negative and CTC negative. Risk of recurrence was similar for patients who were ctDNA positive and CTC negative vs those who were ctDNA negative and CTC positive.

Taken together, the combination of ctDNA and CTCs was associated with increased sensitivity and discriminatory capacity; however, statistically adding CTCs into multivariate models of ctDNA was not associated with improved goodness of fit. This outcome possibly comes from the limited sample size and short duration of follow-up.

Discussion

These results demonstrate that patients with TNBC at high risk of relapse due to an incomplete pathologic response after neo-

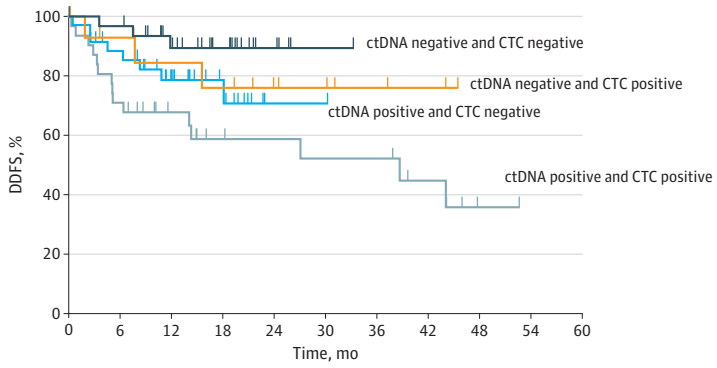
Figure 1. Survival of Study Patients With vs Without Circulating Tumor DNA (ctDNA)



A, Distant disease–free survival (DDFS) (median, 32.5 months vs not reached; hazard ratio [HR], 2.99; 95% CI, 1.38-6.48; P = .006). B, Disease-free survival (DFS) (median, 22.8 months vs not reached; HR, 2.67; 95% CI, 1.28-5.57; P = .009). C, Overall survival (OS) (median, not reached vs not reached; HR, 4.16; 95% CI, 1.66-10.42; P = .002).

Figure 2. Survival of Study Patients With vs Without Circulating Tumor DNA (ctDNA) and Circulating Tumor Cells (CTCs)

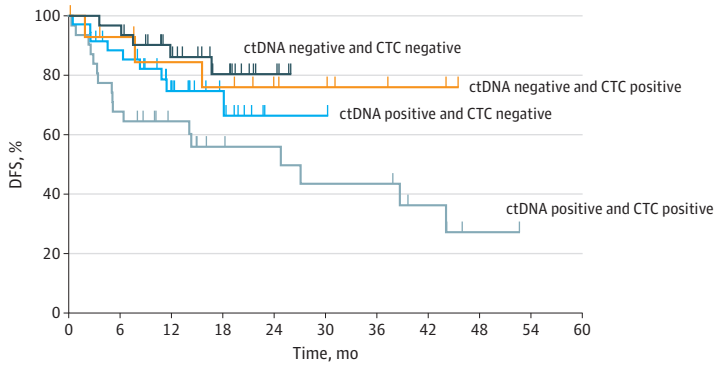
A Association of ctDNA and CTCs with DDFS



No. at risk

ctDNA negative and CTC negative	31	30	22	14	5	1	0	0	0	0	0
ctDNA negative and CTC positive	15	12	10	9	6	5	3	2	0	0	0
ctDNA positive and CTC negative	35	29	18	10	1	1	0	0	0	0	0
ctDNA positive and CTC positive	31	20	14	9	8	8	5	1	0	0	0

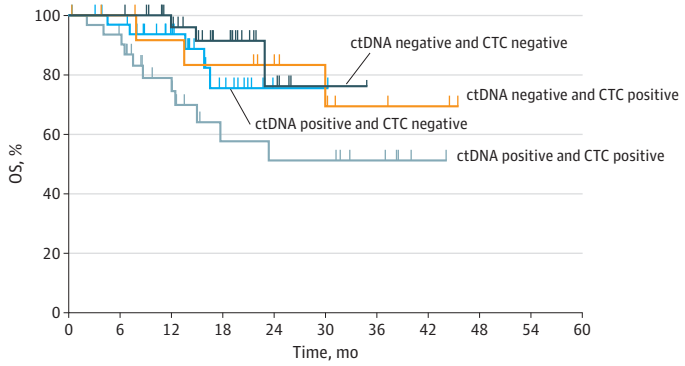
B Association of ctDNA and CTCs with DFS



No. at risk

ctDNA negative and CTC negative	31	30	21	12	4	0	0	0	0	0	0
ctDNA negative and CTC positive	15	12	10	9	6	5	3	2	0	0	0
ctDNA positive and CTC negative	35	29	17	9	1	1	0	0	0	0	0
ctDNA positive and CTC positive	31	20	14	9	7	7	4	1	0	0	0

C Association of ctDNA and CTCs with OS



No. at risk

ctDNA negative and CTC negative	31	31	24	15	5	1	0	0	0	0	0
ctDNA negative and CTC positive	15	13	11	10	7	5	3	2	0	0	0
ctDNA positive and CTC negative	35	31	21	10	1	1	0	0	0	0	0
ctDNA positive and CTC positive	31	28	18	9	8	8	5	1	0	0	0

A, Distant disease-free survival (DDFS) (median, 32.5 months vs not reached; hazard ratio [HR], 5.29; 95% CI, 1.50-18.62; *P* = .009). B, Disease-free survival (DFS) (median, 20.8 months vs not reached; HR, 3.15; 95% CI, 1.07-9.27; *P* = .04). C, Overall survival (OS) (median, not reached vs not reached; HR, 8.60; 95% CI, 1.78-41.47; *P* = .007).

adjuvant chemotherapy can be risk stratified with MRD. The results here are significant from an effect-size standpoint and remain highly significant after consideration of multiple clinical variables. These results add substantially to the prior body

of literature because this study provides one of the largest data sets to date, to our knowledge, and is a preplanned secondary analysis of a prospective randomized clinical trial.

The postneoadjuvant setting is one that is in need of marked improvements, especially for the subgroup of patients with residual disease. Our findings now support using MRD as a major stratification variable in all clinical trials to be conducted in this setting. In addition, the ability to sequence ctDNA broadly for important gene variations affords the possibility of not only uncovering an ultra-high-risk population for relapse but also revealing drug targets. Perhaps equally important, if the results from the group of patients who are ctDNA negative and CTC negative hold, this may be a subgroup in which the patients do not benefit from additional therapy, and this may be an ideal place to study novel de-escalation strategies. At the present time, we would discourage the use of MRD as a marker for relapse or to guide therapy in routine clinical practice because there is no evidence that early detection improves outcomes.

Limitations

This study has some limitations, including the potential interaction with the type of therapy delivered in the postneoadjuvant setting. Although not controlled for in this analysis, there was an equal distribution of testing across both groups and an equal distribution of ctDNA and CTC positivity and negativity across both groups. Another limitation is the relatively short duration of follow-up. This concern is minimized by the well-established early relapse and the infrequent late relapse seen in this population.

Conclusions

The strength of these findings, along with the prior body of literature,[2,3,15] now supports the routine use of this technology for proper risk stratification across clinical trials in the curative setting. Future trials will determine if genomically guided therapeutic interventions in patients who have molecular MRD can improve outcomes. This concept will be the centerpiece of our planned successor trial to BRE12-158: the PERSEVERE trial, whereby patients with TNBC with ctDNA positivity after surgery will be assigned to receive a targeted agent matched to the patients' plasma sequencing results.

ARTICLE INFORMATION

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Author Contributions: Drs Radovich and Schneider had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Dr Radovich and Mr Jiang contributed equally to this work.
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Supervision: Radovich, Nanda, Lynce, Mahtani, Albacker, Gregg, Badve, Savran, Schneider.

Conflict of Interest Disclosures: Dr Radovich reported serving as an advisor for LifeOmic; and having stock ownership in LifeOmic, Macrogenics, Immunomedics, Arqule, and Tyme Technologies outside the submitted work. Dr Falkson reported receiving grants from TBCRC during the conduct of the study. Dr Lynce reported receiving grants from Indiana University during the conduct of the study; and grants from Inivata, Tesaro, Regeneron, Immunomedics, Calithera, and Chugai; grants and personal fees from Pfizer; receiving grants from and serving as a consultant/advisor for BMS; serving as a consultant/advisor for AstraZeneca; and receiving personal fees from ASCO, outside the submitted work. Dr Isaacs reported receiving grants from the National Cancer Institute during the conduct of the study; and personal fees from Genentech, Pfizer, Novartis, AstraZeneca, PUMA, and Seattle Genetics; and grants from Tesaro outside the submitted work. Dr Paplomata reported receiving grants from Hoosier Cancer Research Network during the conduct of the study; and grants and personal fees from Novartis; grants and meals from Genentech and Merck; personal fees from Pfizer, R-Pharm, and Mylan; meals from Tesaro and Amgen; grants from Corcept, Seattle Genetics, and Cascadian outside the submitted work. Dr Mahtani reported receiving personal fees for serving as a consultant/advisor for Agendia, Biotheranostics, Daiichi, Eisai, Genentech, Pfizer, Lilly, Novartis, Seattle Genetics, and Puma outside the submitted work; and reported research support from Genentech to her institution. Dr Thompson reported serving as a consultant/advisor for Syapse, UpToDate, and Doximity; and personal fees from VIA Oncology, Adaptive, GSK, Takeda, and Celgene outside the submitted work. Mr Pavlick reported receiving personal fees from Foundation Medicine and F. Hoffmann-La Roche AG outside the submitted work. Dr Albacker reported receiving personal fees from Foundation Medicine Inc and Roche Holding AG outside the submitted work. Dr Gregg reported receiving personal fees from Foundation Medicine, AstraZeneca, BMS, Novartis, and Roche; and grants from AstraZeneca outside the submitted work. Dr Chen reported being currently employed at Foundation Medicine Inc. Dr Ballinger reported receiving personal fees from Novartis and Medscape outside the submitted work. Dr Chang reported receiving grants from Tom Hurvisand the McKinley Educational Foundation; and personal fees from Savran Technologies Inc during the conduct of the study; stock ownership from Savran Technologies Inc outside the submitted work; and having a patent to 10,335,790 issued, a patent to 10,207,267 issued, a patent to EP2694965B1 issued, a patent to 9,494,557 issued, and a patent to 9,500,625 issued. Mr Zhong reported receiving personal fees from Savran Technologies Inc during the conduct of the study. Dr Savran reported receiving grants from McKinley Educational Foundation and Tom Hurvis during the conduct of the study; stock ownership from Savran Technologies Inc outside the submitted work; and having a patent to 10,335,790 issued, a patent to 10,207,267 issued, a patent to EP2694965B1 issued, a patent to 9,494,557 issued, and a patent to 9,500,625 issued. No other disclosures were reported.

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