Impact of DNA Repair Deficiency Signature on Outcomes in Triple Negative Breast Cancer (TNBC) Patients Treated with AC Chemotherapy (SWOG S9313)

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Abstract #529

Background

- There is a critical need to identify and validate biomarkers of response and resistance to adjuvant chemotherapy for TNBC.
- In preliminary studies, deficiency in DNA damage response (DDR) and repair pathways have been reported in TNBC patients and may impact response to chemotherapy.[1]
- We report on the prognostic impact of three biomarkers in a large cohort of early stage TNBC patients who were treated with adjuvant doxorubicin (A) and cyclophosphamide (C) on S9313 .

Aim

- To investigate DNA damage response deficiency (DDRD) molecular signature, *BRCA1* mRNA expression and tumor infiltrating lymphocytes (TILs) as prognostic markers in TNBC patients treated with adjuvant AC.

Methods

- SWOG protocol S9313 accrued 3,125 women with early stage breast cancer to two alternative dose schedules of AC with no difference in outcomes between the two arms.[2]
- 425 patients with centrally determined TNBC (ER and PR Allred score of 0, HER2 negative per 2013 ASCO-CAP guidelines) were identified
- The DDRD signature score (Almac Diagnostics) is based on the expression of 44 genes involved in immune response, cell proliferation, and metabolism.
- Total RNA was extracted from pre-treatment FFPE breast tumor tissue, amplified, fragmented , labeled, and hybridized to microarrays.
- DDRD scores were derived from microarrays imaged using the GeneChip® Scanner 3000 (NuGEN Technologies) and were classified by tertiles . High DDRD tertile indicates presence DNA repair deficiency and low tertile indicates DNA repair proficiency
- *BRCA1* mRNA quantification was performed on the nCounter® platform (NanoString Technologies). Raw counts were normalized to internal controls and to reference transcripts using nSolver Analysis Software 3.0.
- Histopathologic determination of TILs density was jointly performed by two pathologists (S.B., Y.G.) who were blinded to outcome information, on a single H&E stained invasive tumor section. Density is reported as a percentage estimate in increments of 10.[3,4,5]
- The markers were tested for prognostic effect on DFS and OS using a Cox regression model with adjustment for randomized treatment assignment and nodal status.

Table 1: Patient characteristics and outcomes by DDRD score

	All 425 patients	DDRD determined & included n=302	DDRD Tertile 1 N=100	DDRD Tertile 2 N=102	DDRD Tertile 3 N=100
Definition		Included if tumor content ≥50%	DDRD 0.0000 – 0.2949	DDRD 0.2950 – 0.4619	DDRD 0.4620 – 1.0000
Mean age in years (range)	45.6 (22-74)	45.3 (22-74)	46.3 (25-74)	45.1 (22-73)	44.6 (27-68)
Nodal status					
Negative	285 (67.1%)	201 (66.6%)	63 (63.0%)	71 (69.6%)	67 (67.0%)
Positive	140 (32.9%)	101 (33.4%)	37 (37.0%)	31 (30.4%)	33 (33.0%)
Randomized treatment					
Combined AC	220 (51.8%)	160 (53.0%)	53 (53.0%)	54 (52.9%)	53 (53.0%)
Sequential AC	205 (48.2%)	142 (47.0%)	47 (47.0%)	48 (47.1%)	47 (47.0%)
5-year DFS (95% CI)	74.3% (69.8%-78.2%)	73.8% (68.4%-78.4%)	63.0% (52.8%-71.6%)	76.3% (66.7%-83.4%)	82.0% (73.0%-88.3%)
5-year OS (95% CI)	83.0% (79.1%-86.2%)	82.1% (77.2%-86.0%)	76.0% (66.4%-83.2%)	83.2% (74.3%-89.2%)	87.0% (78.7%-92.2%)
10-year DFS (95% CI)	66.3% (61.6%-70.7%)	65.8% (60.1%-70.9%)	54.6% (44.3%-63.8%)	66.7% (56.4%-75.0%)	75.9% (66.2%-83.1%)
10-year OS (95% CI)	74.1% (69.6%-78.0%)	73.4% (68.0%-78.1%)	65.5% (55.2%-74.0%)	73.8% (63.9%-81.4%)	80.8% (71.6%-87.3%)

Results

- For 425 TNBC at a median follow-up of 12.6 years, there were 166 DFS and 129 OS events (5-year DFS and OS = 74% and 83%, respectively).
- The DDRD signature was evaluated in 89.4% (380/425), but only 302 samples (71.1%) met criterion of ≥ 50% tumor content for marker inclusion. DFS did not differ between those included or those excluded from analysis (p=0.95)
- Higher DDRD score modeled as a continuous variable was associated with improved DFS (p<0.001) and improved OS (p=0.004)
- DDRD tertiles were positively associated with DFS both as categories (p=0.0019) and as a trend (p=0.001). OS showed similar associations categorically (p=0.0138) and as a trend (p=0.004). (Figure 1 and Table 1)
- Association of DDRD score and outcome was not modified by nodal status or treatment
- TIL density was successfully determined in 99.5% (423/425) of samples. TIL density was positively associated with DFS. For every 10% increase in TILS, HR = 0.87; 95% CI 0.78-0.96; p = 0.008) and OS (HR = 0.82; 95% CI 0.73-0.93; p = 0.002). (Figure 2)
- *BRCA1* mRNA expression results are available for 395/425 (93%) of samples, and was not associated with DFS (p=0.21) or OS (p=0.10). (Figure 3)
- DDRD score and TIL density were moderately correlated (Pearson r = 0.62). (Figure 4)

Figure 1: DFS and OS by DDRD Tertiles

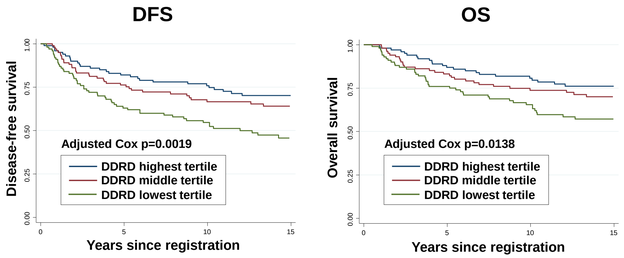


Figure 2: DFS and OS by TIL density

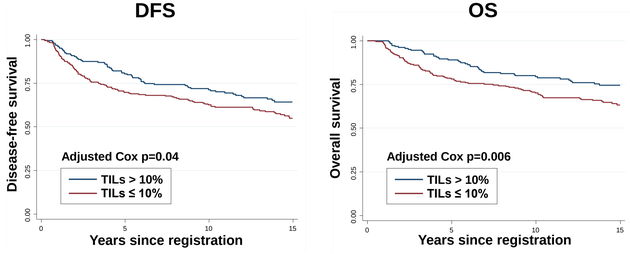


Figure 3: DFS and OS by *BRCA1* mRNA expression

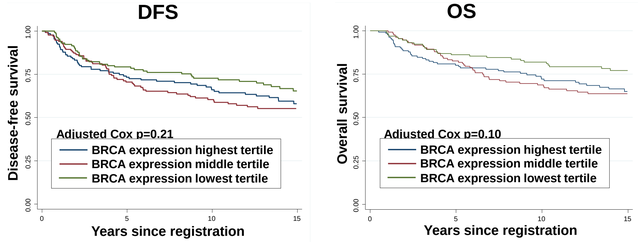
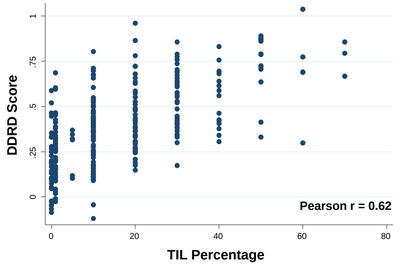


Figure 4: Association of DDRD score with TIL density



Conclusions and Discussion

- In this correlative study we demonstrate that high DDRD scores are associated with good prognosis in TNBC patients treated with adjuvant AC.
- Compared to patients with lowest tertile DDRD score, patients with highest tertile DDRD score had a 54% lower risk of recurrence/death when treated with adjuvant AC.
- Previous studies have reported that TILs are associated with chemotherapy response and good prognosis in TNBC[3,4,5]. Our findings in the current study are consistent with previous reports.
- Prognostic and predictive impact of the DDRD signature should be evaluated in other TNBC cohorts. With appropriate validation the DDRD signature has potential to be used as selection criterion to identify TNBC patients who will either receive significant benefit from AC or have suboptimal outcomes with AC.
- Molecular drivers of tumors with low DDRD scores should be investigated further in order to identify better treatment options for this group.

References

1. Mulligan et al J Natl Cancer Inst 2014; 2. Linden et al J Clin Oncol 2007; 3. Adams et al J Clin Oncol 2014; 4. Denkert et al J Clin Oncol 2010; 5. Loi et al J Clin Oncol 2013.

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