San Antonio Breast Cancer Symposium® - December 10-14, 2019

Using multi-omic profiling to unravel the complexity of triple-negative breast cancer

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Background

- Triple-negative breast cancer (TNBC) is a heterogeneous disease.
- Studies have classified TNBCs into different subgroups based on:
  - mutational profile
  - patterns of gene expression
  - expression of protein markers
  - degree of immune cell infiltration
- Although TNBC heterogeneity has been characterized at each of these levels individually, how variation at one level is associated with differences at other levels is poorly understood.
- In this study, we performed "multi-omic" profiling on a cohort of TNBCs in order to determine how various DNA, RNA, protein, and immunologic parameters are correlated.

Methods

Formalin-fixed, paraffin embedded TNBC samples* (n=95)

Breast pathologists scored sTILs (according to guidelines from the International TILs Working Group) and IHC staining (intensity and % of cells stained). Antibodies were: PD-L1 clone 405.9A11 (Cell Signaling Technology); AR clone AR441 (Dako); RB clone G3-245 (BD Biosciences).

Breast Cancer 360 (BC360) panel (Nanostring) includes algorithms to score various signatures (e.g. TNBC subtype, immune signatures, differentiation, etc).

DNA sequencing results were available from 68/95 (72%) of cases.

H and E section
Stromal tumor-infiltrating lymphocytes (sTILs, %)

Immunohistochemistry
Androgen receptor (AR)
Retinoblastoma protein (RB)
Programmed death-ligand 1 (PD-L1)

RNA extraction from tumor-rich regions
Expression of 776 genes using nCounter "BC360" assay

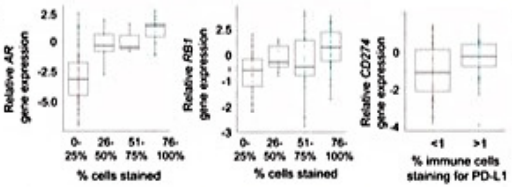
DNA extraction
"Oncopanel" assay: in-house sequencing panel for coding regions of ~500 genes

Samples were acquired during pre-screening for an ongoing clinical trial (NCT03130439) partially funded by Eli Lilly. All patients provided consent for the profiling in this study.

Characteristics of study samples

Tumor site (n=95)	Primary	67%
	Regional nodal	4%
	Distant nodal or visceral	28%
Stromal TILs (n=93)	<10%	53%
	10-49%	32%
	>50%	14%
RB staining (n=95)	Positive (>50% tumor cells)	55%
AR staining (n=95)	Positive (>1% tumor cells)	36%
Stromal PD-L1 (n=94)	staining in >1% mononuclear cells	37%
Tumor cell PD-L1 (n=94)	staining in >1% tumor cells	29%
PAM50 subtype (n=90)	Basal	80%
	HER2-enriched	17%
	Luminal B	3%
TNBC subtype (n=91)	Basal-like immune activated (BLIA)	80%
	Basal-like immunosuppressed (BLIS)	7%
	Mesenchymal (MES)	8%
	Luminal androgen receptor (LAR)	4%
DNA alterations (n=68)	TP53 alterations	80%
	BRCA1/2 mutations	21%
	RB1 alterations	31%
	PI3K pathway alterations	46%

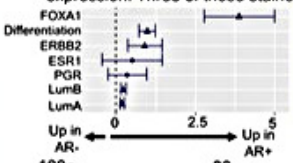
Biomarker protein-RNA correlations



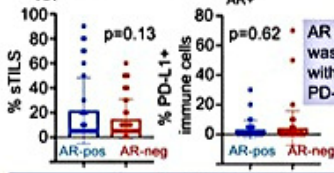
The protein expression of AR, RB1, and PD-L1 was well correlated with expression of the genes encoding them.

Analysis by AR status

- 36% of tumors stained AR-positive (>1% of tumor cells). 65% of these stained RB-positive (>50% tumor cells).
- 75% of AR-positive tumors were *RB1* wild-type (n=27).
- Only 4 tumors (4% of total) classified as "LAR" by gene expression. Three of these stained AR+.



AR+ tumors showed upregulated expression of genes and signatures reflecting luminal biology and differentiation.

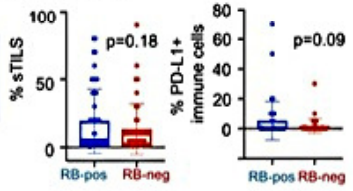


AR status by IHC was not associated with TIL infiltration or PD-L1 status

Analysis by RB status

- 55% of tumors stained RB-positive (>50% of tumor cells). 42% of these were AR+ by IHC. There was no association between RB status by IHC and TNBC subtype.
- 30% of tumors had an *RB1* alteration. 48% of these stained positive for RB (>50% tumor cells).
- 70% of tumors were *RB1* wild-type. 60% of these stained positive for RB (>50% of tumor cells).

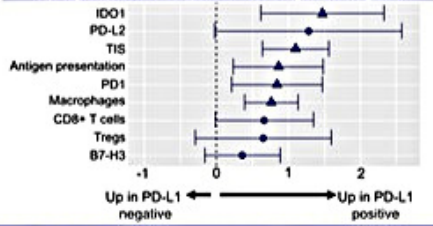
RB status by IHC was not associated with TIL infiltration or PD-L1 status



Analysis by PD-L1 status

- There was a significant correlation between stromal TIL infiltration and fraction of PD-L1+ immune cells in tumors (r=0.582, p<0.001).
- PD-L1 status (<1 vs >1% immune cells stained did not correlate with TNBC subtype (by gene expression).

PD-L1+ (>1% of immune cells) tumors show upregulation of multiple immune signatures/genes



Analysis by genomic alterations

- PI3K pathway altered tumors (*PIK3CA, PTEN, AKT1, AKT2, AKT3*) showed significantly higher expression of *AR* (p<0.001) and *FOXA1* (p=0.004*).
- *BRCA1/BRCA2* mutant tumors (somatic) showed a significantly higher "tumor inflammation signature" (TIS) score by gene expression (p=0.014*).
- *RB1* altered tumors showed higher proliferation indices by gene expression (p=0.068*). (* - unadjusted p values)

Summary

- Neither RB nor AR status by IHC was associated with TIL infiltrate or PD-L1 status.
- RB status by IHC did not associate with particular TNBC subtypes by gene expression.
- RB IHC is a poor surrogate for *RB1* alteration status.
- AR-positive tumors were no more likely to be RB-positive by IHC or *RB1* wild-type.