Abstract 031: Tumor infiltrating lymphocyte (TIL) recruitment after peri-lymphatic IRX-2 cytokine immunotherapy in resectable breast cancer and head and neck carcinoma

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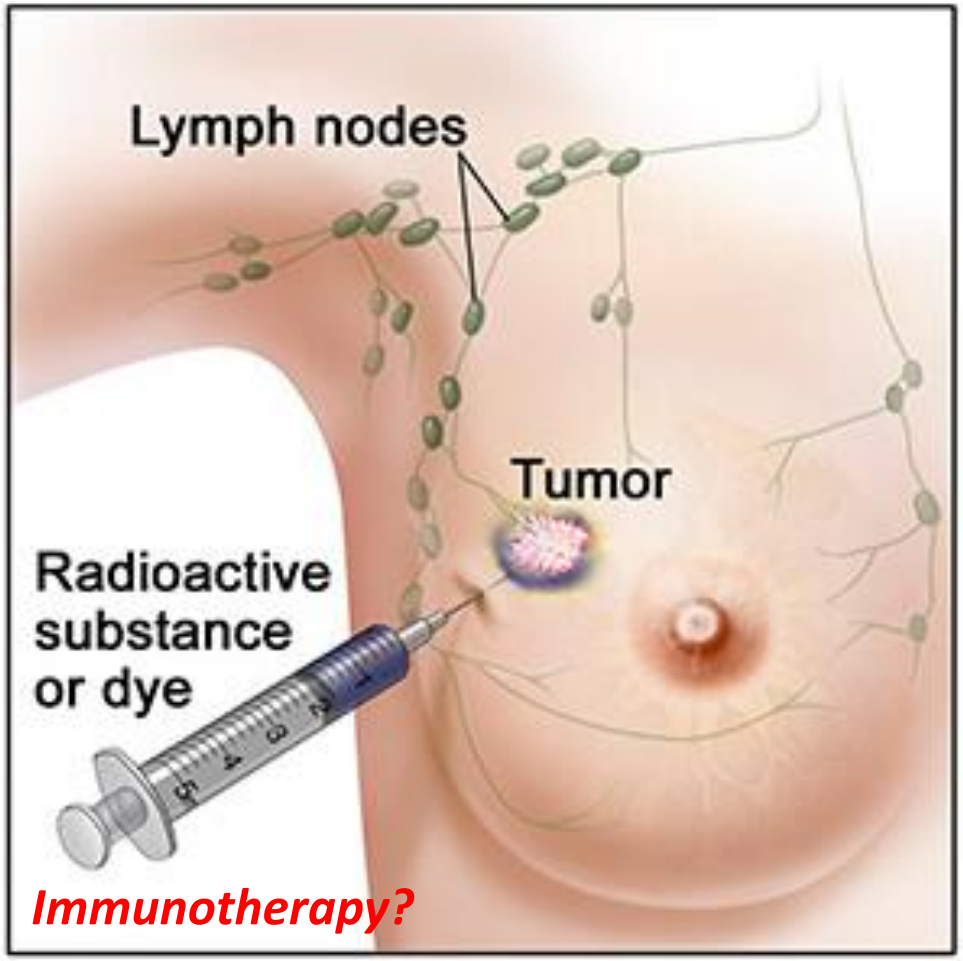
Introduction

- ~40,000 USA deaths from breast cancer recurrence annually[1]
- TIL infiltration predicts freedom from recurrence and chemotherapy response[2]
- Anti-PD-1/L1 clinical activity in breast cancer, however activity is higher in PD-L1+ tumors[3]
- A locoregional immunotherapy approach may enhance response while minimizing toxicity



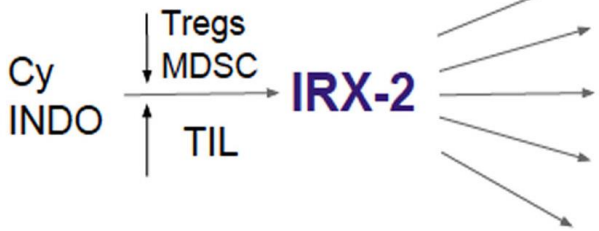
Hypothesis: Cytokine-based immunotherapy could be administered to regional lymphatics via subcutaneous injection, and may enhance TILs without added toxicity

Ref: [1]web: https://seer.cancer.gov/; [2]Stanton SE, et al, JAMA Oncol 2016 [3]Schmid P, et al, NEJM 2018

Treatment and Clinical Trial Schematic

a

b



Cytokine	Concentration
IL-2	4000-8000pg/mL
IFNg	1000-3800pg/mL
IL-1b	300-1400pg/mL
TNFa	1000-4300pg/mL
Also contains measurable amounts of: IL-6, IL-8, GM-CSF, G-CSF	

Biologic Effects

Enhanced T-cell Activation[1]
Enhanced DC Maturation[1]
Increased B-cells[3]
Increased NK-cells[2]
Increased T-cells[3]
Increased Antigen Presentation[1]
Inhibition of Apoptosis[4]

Legend: **Cy**=cyclophosphamide; **Sx**=surgery; **MDSC**=myeloid-derived suppressor cells

Components of therapy: Low-dose cyclophosphamide (Cy, 300mg/m2); peri-areolar IRX-2 SC injections (230 IU cytokine/d x 10); oral indomethacin (oral COX1/2 inhibitor)

Ref: *[1]Egan et al.., 2007; [2]Whiteside et al., 2011; [3]Wolf et al., 2018 [4]Czystowska et al., 2009*

Clinical Trial Objectives

Primary:

- To evaluate feasibility of pre-operative peri-lymphatic immunotherapy

Secondary:

- To evaluate for increases in stromal TILs[1]

Exploratory:

- Pre/post-treatment PD-L1 expression
- Pre/post intratumoral immune cell phenotype/activation
- Peripheral blood treatment effects

Ref: [1]Salgado R, et al, Ann Oncol 2015

Results: Subject Demographics

- Median age 55y (range 40-78)
- Majority of tumors are HR+HER2- (69%, n=11/16)
- Minority are HER2+ (25%, n=4/16)
- n=1 stage I TNBC tumor
- 50% grade I/II, 50% grade III
- 50% node-positive (n=8/16)

Clinical trial subjects

Patient ID	Age	Histology	Grade	Ki67%	Tumor size
IRXB-001	78	HR+Her2-	2	11%	1.9cm
IRXB-002	58	HR+Her2-	2	19%	2.4cm
IRXB-003	40	HR+Her2-	2	17%	3.0cm
IRXB-004	64	HR-Her2+	3	75%	4.2cm
IRXB-005	48	HR+Her2-	1	7%	1.7cm
IRXB-006	62	HR-Her2+	3	73%	2.7cm
IRXB-007	45	HR+Her2-	3	55%	3.7cm
IRXB-008	54	HR+Her2-	3	50%	2.1cm
IRXB-009	56	HR+Her2-	2	11%	0.7cm
IRXB-010	46	HR+Her2-	2	33%	1.8cm
IRXB-011	52	HR+Her2+	3	38%	1.5cm
IRXB-012	52	HR+Her2+	3	12%	2.1cm
IRXB-013	59	HR+Her2-	3	87%	2.2cm
IRXB-014	45	HR+Her2-	1	N/A	2.2cm
IRXB-015	61	HR+Her2-	2	30%	1.9cm
IRXB-016	66	TNBC	3	95%	1.0cm

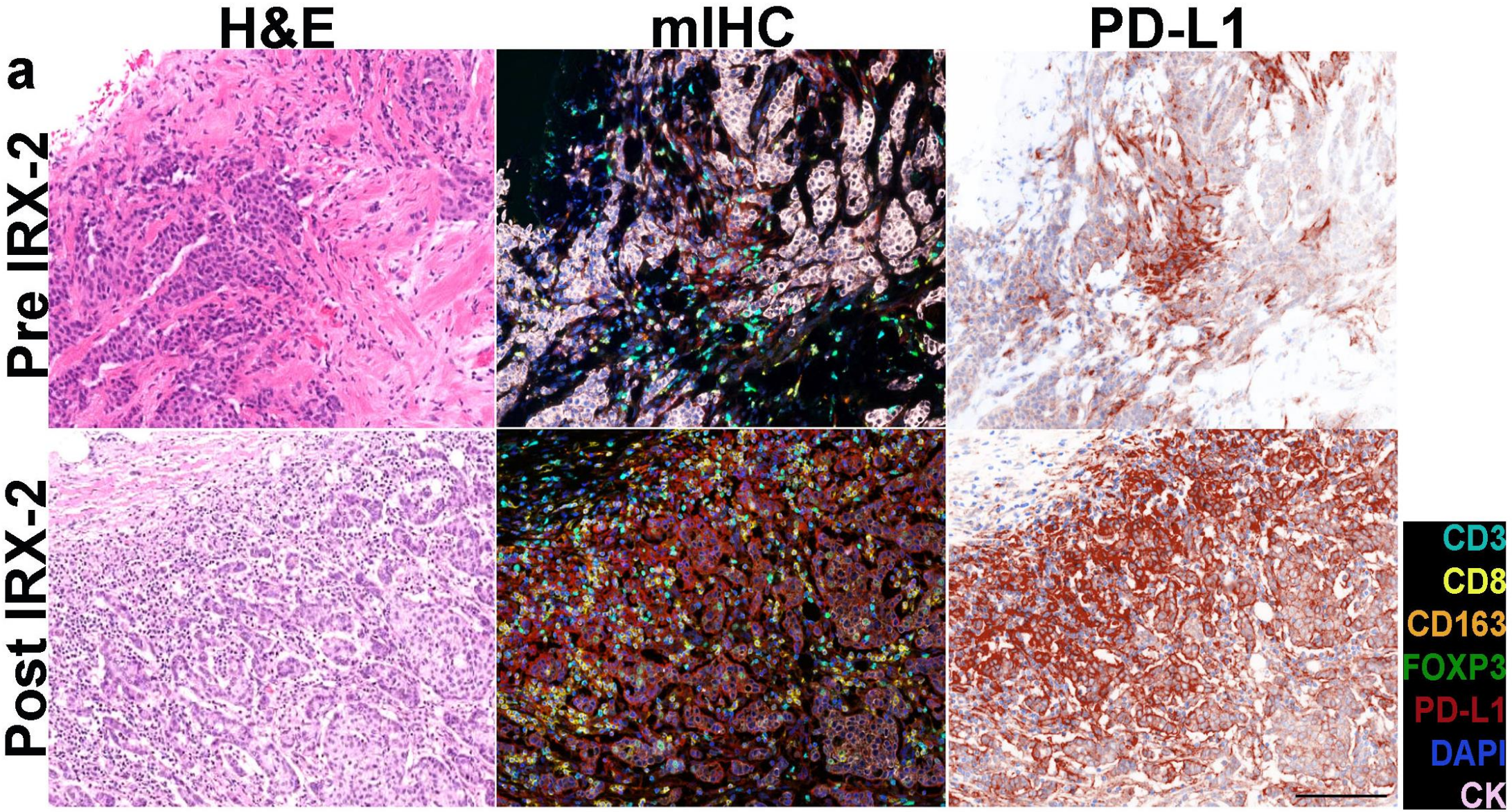
Results: Feasibility

- No treatment-related grade III/IV toxicities;
- One treatment-unrelated transient grade III syncope in subject with prior history of vasovagal episodes
- Nausea and abdominal cramping associated with indomethacin (50%, 19%)
- Transient injection site erythema/bruising (50%, 44%)
- Minimal pain (19%), none requiring topical anesthesia

CTCAE 4.0 Toxicities occurring in >15% of subjects

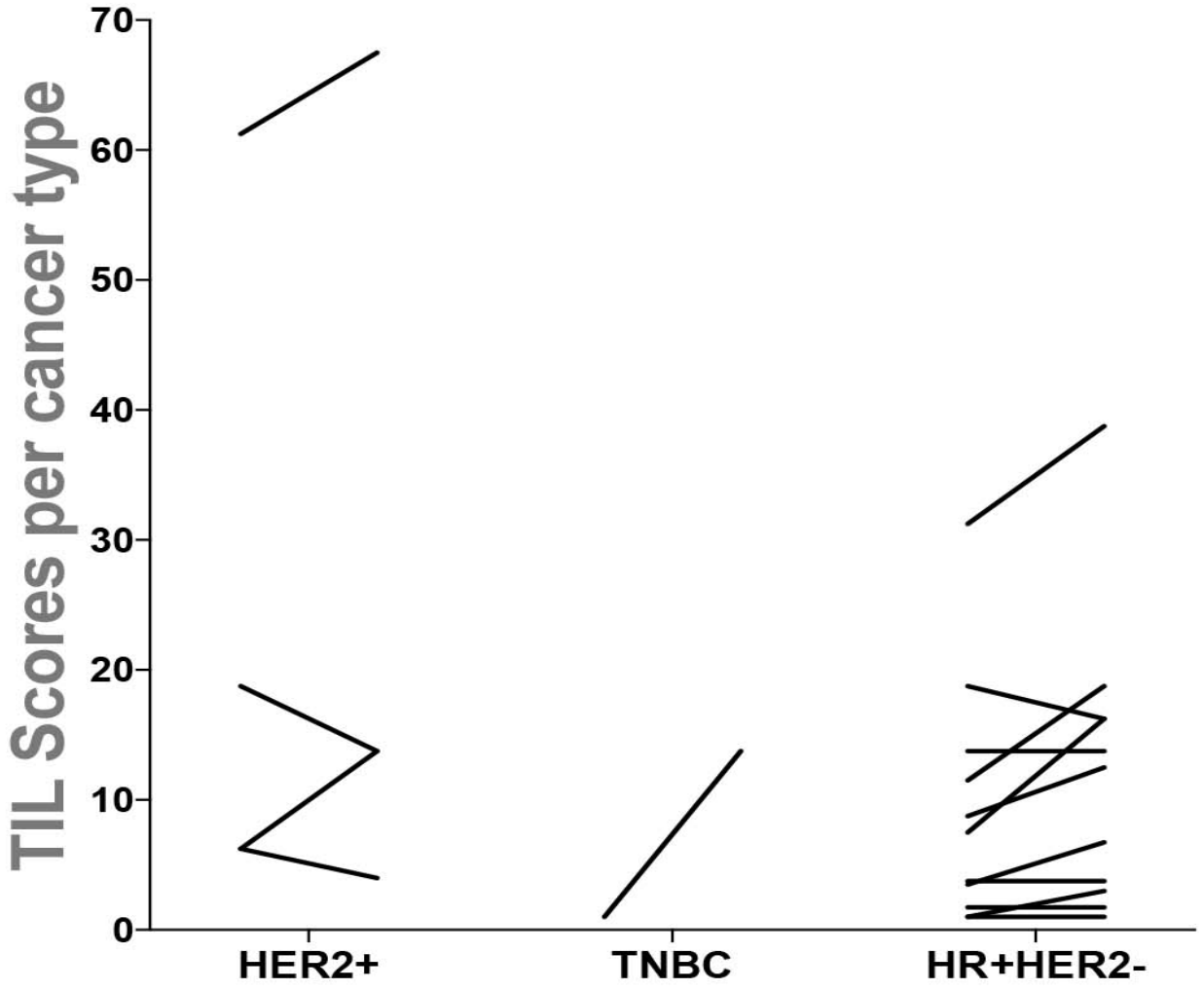
Toxicity	Grade I/II, any attribution (%)	Grade I/II, Cy (%)	Grade I/II, Indo (%)	Grade I/II, IRX-2 (%)
Nausea	13 (81%)	3 (19%)	8 (50%)	
Bruising	8 (50%)			7 (44%)
Fatigue	9 (56%)	5 (31%)		
Injection site reaction	8 (50%)			8 (50%)
Abdominal cramping/bloating	4 (25%)		3 (19%)	
Increased ALT/AST/AlkP	4 (25%)			
Anemia	4 (25%)	1 (6%)	1 (6%)	
Headache	5 (31%)	1 (6%)		
Hypokalemia	4 (25%)			
Anorexia	3 (19%)			
Diarrhea	3 (19%)	1 (6%)	1 (6%)	
Flatulence	3 (19%)		2 (13%)	
Injection site pain	3 (19%)			3 (19%)
Vomiting	3 (19%)	2 (13%)	2 (13%)	

Results: TILs analysis



Results: Stromal TIL score

- 2015 San Antonio Working Group method[1]
- sTIL increases in 11/16 subjects
- Mean +1.2-fold relative increase in sTIL score (p=.02, paired t-test)
- Mean +3.1% absolute increase in sTIL (p=.02, paired t-test)
- Intraobserver agreement among 2 blinded pathologists (Cohen's linear kappa=0.53)
- No obvious associations according to histologic type, grade, or Ki67

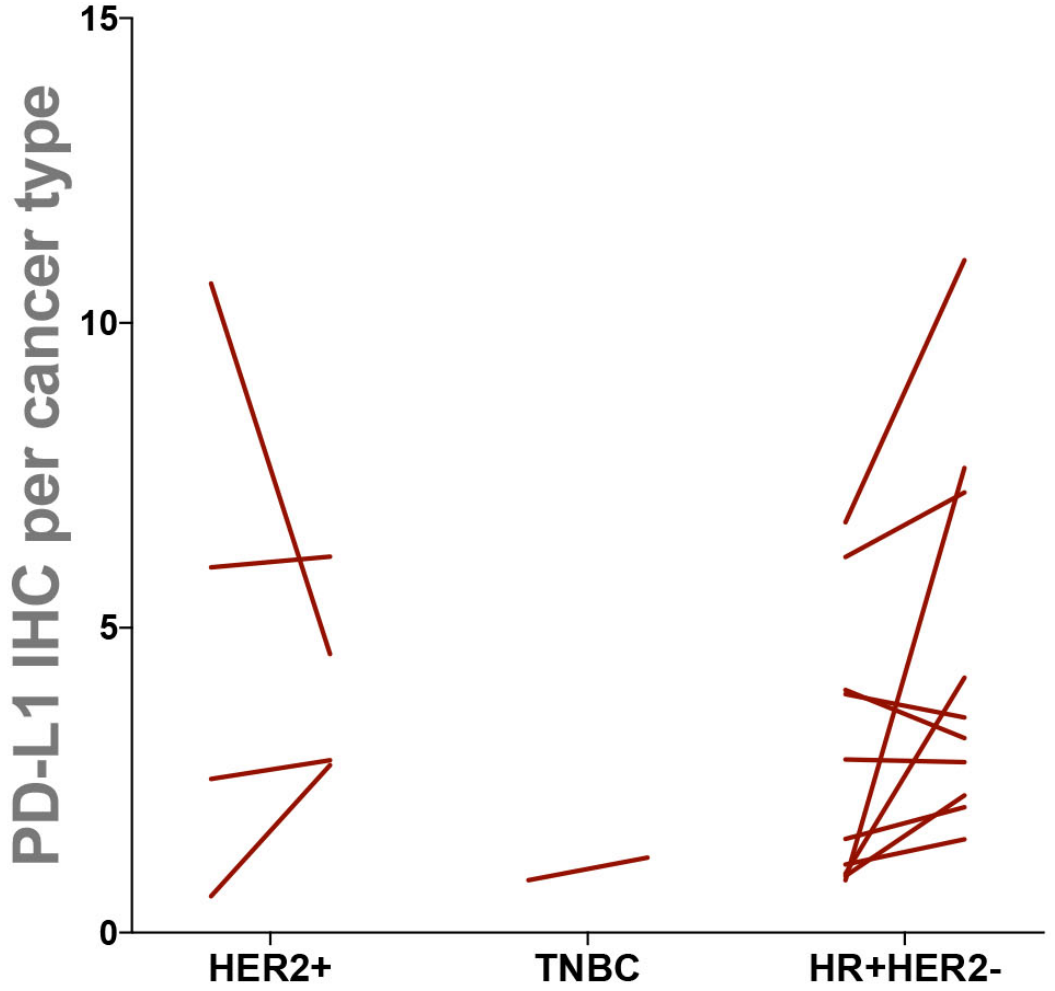


Ref: [1]Salgado R, et al, Ann Oncol 2015

Results: PD-L1 expression

Nanostring mRNA expression

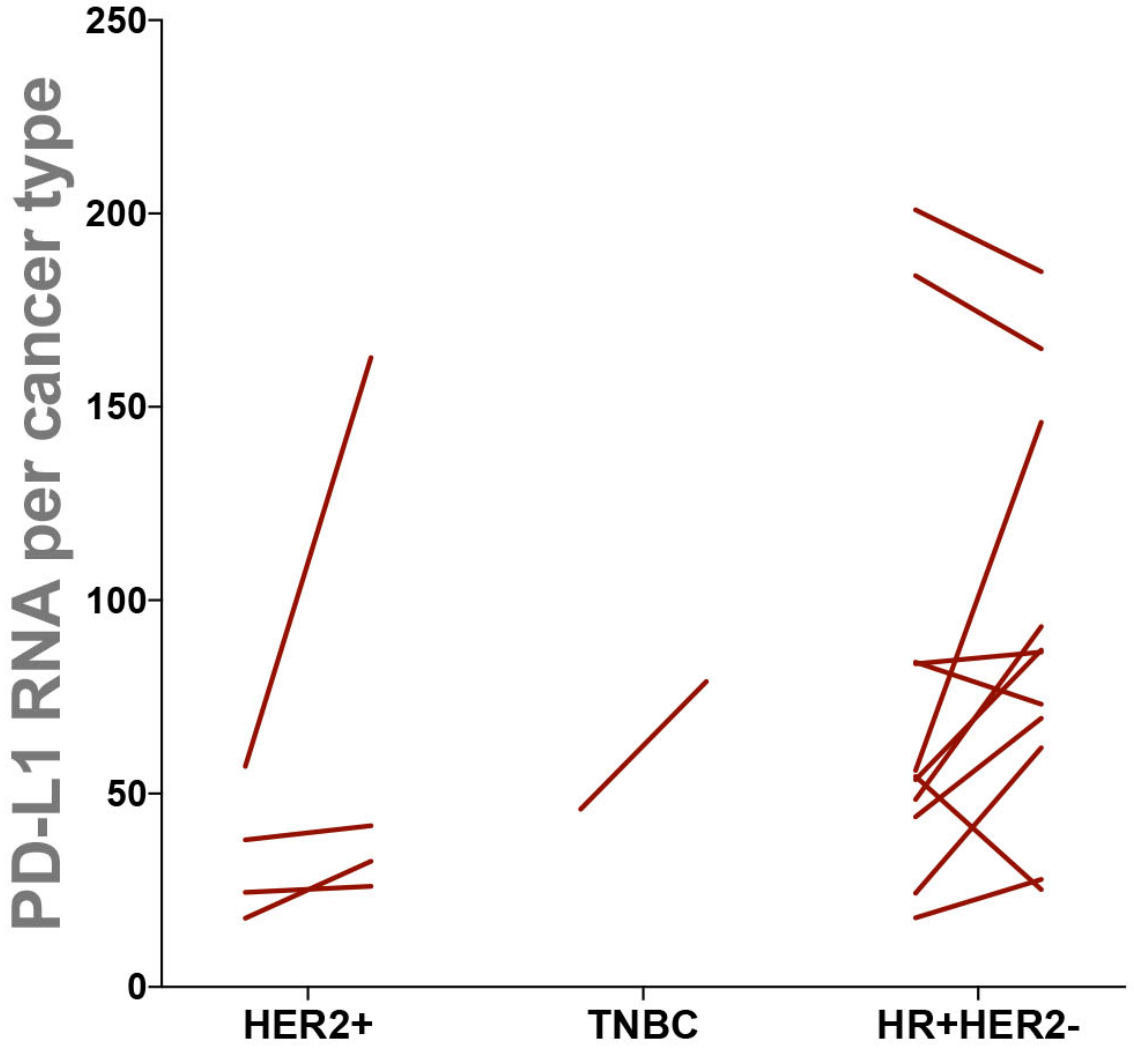
- Increases in PD-L1 mRNA observed in 75% of patients (n=12/16, p=.04, Wilcoxon matched-pairs rank test)
- Mean change mRNA expression +54% (p=.04, paired T-test)
- Increases observed across all histology types



Results: PD-L1 expression

PerkinElmer mIHC

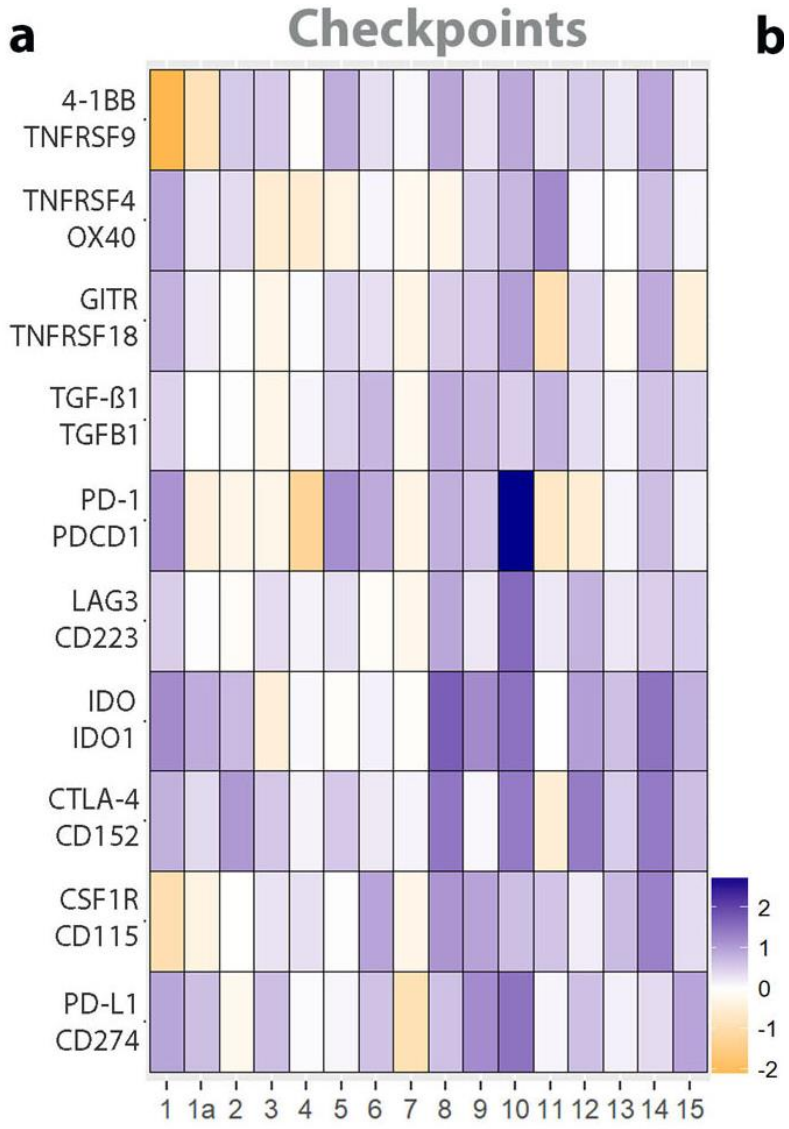
- Increases in mean per-cell PD-L1 fluorescence in 80% of patients (n=12/15, p=.07, Wilcoxon matched-pairs rank test)
- Mean cell change in fluorescence +116% (p=NS)

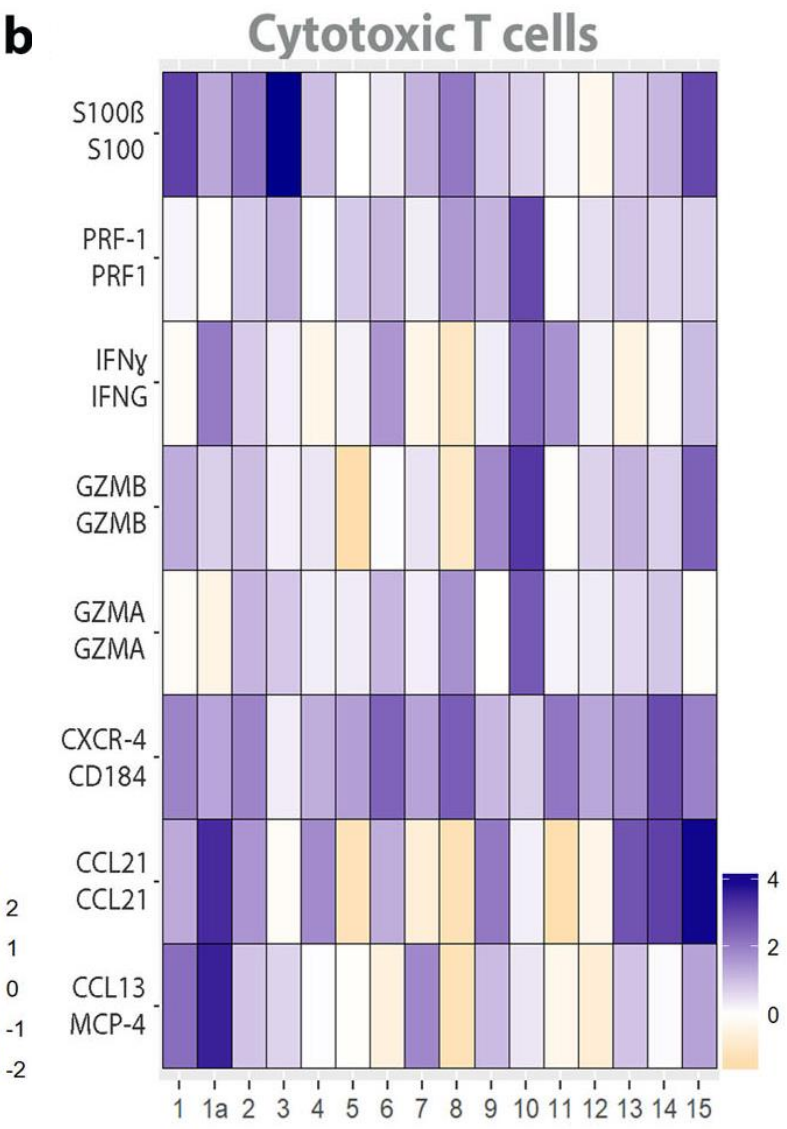


Results: Nanostring immune profile

Relative increases in Nanostring mRNA signatures, including:

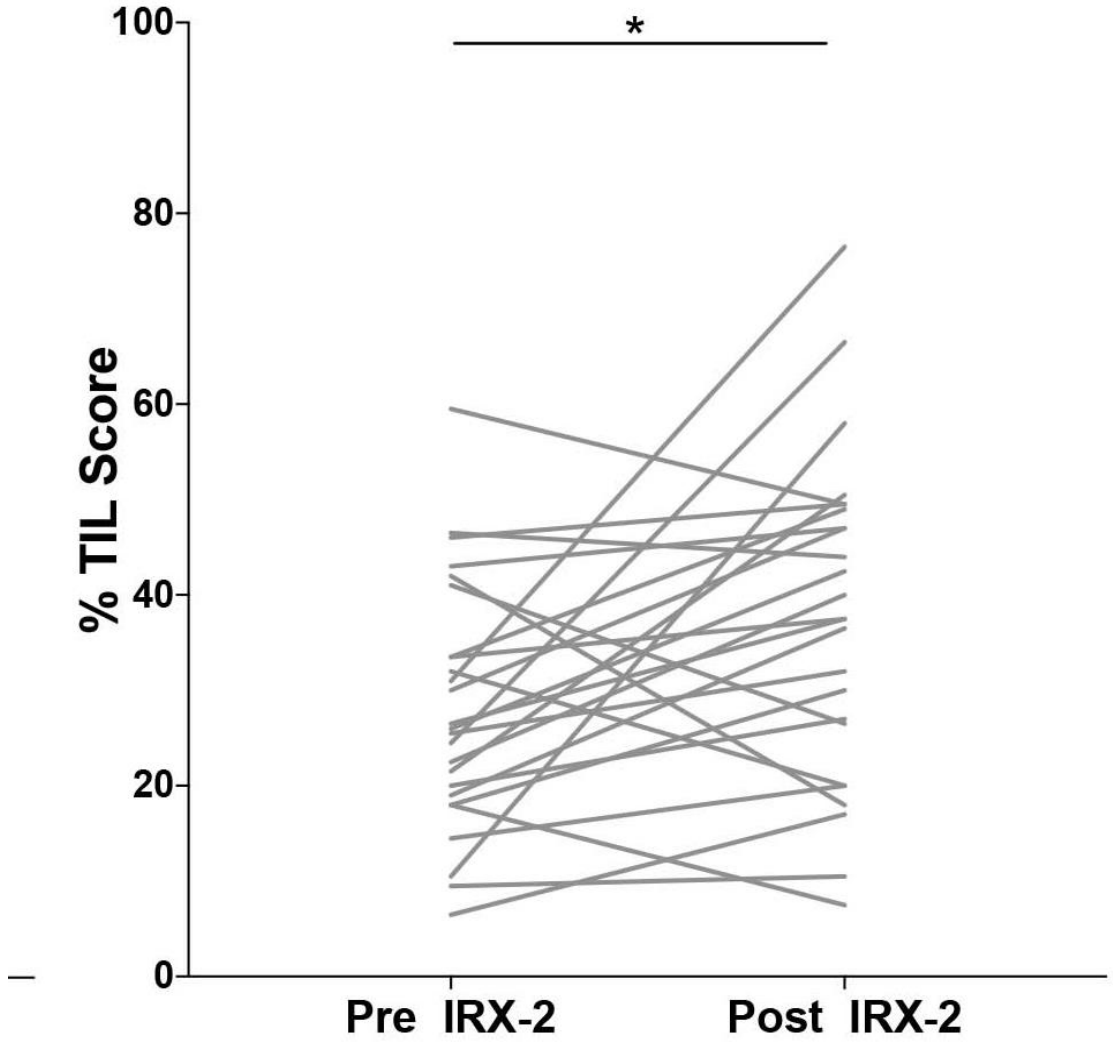
- immune checkpoint markers (OX40, PD-L1, IDO1, CTLA-4)
- leukocyte recruitment signature
- leukocyte markers
- cytotoxic T-cell signature





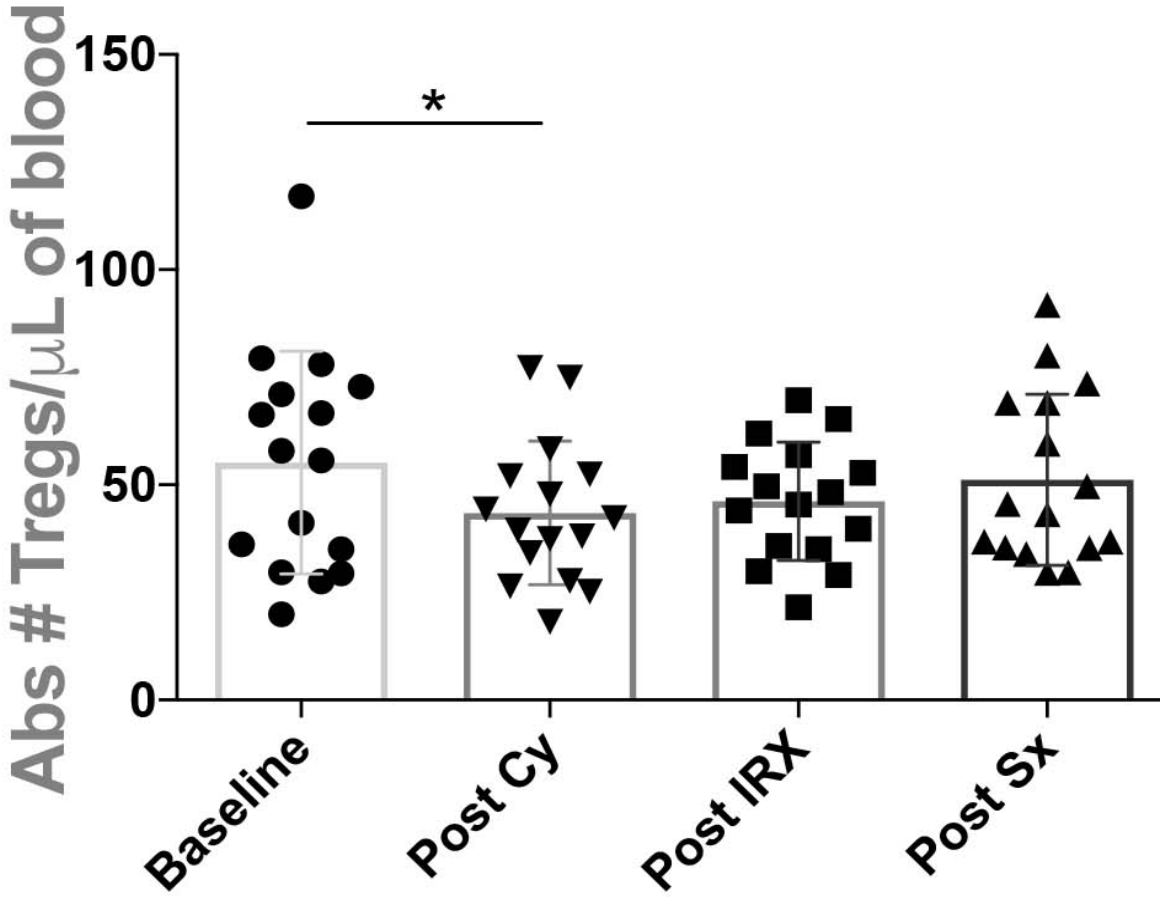
Results: Head and Neck SCC Cohort

- Similar increases in TIL score observed in HNSCC
- Mean 58% relative increase in score (range -57% to +452%, p=.01, paired T-test)
- No changes in PD-L1 mRNA, but higher baseline levels relative to ESBC cohort



Results: Peripheral Blood Effects (flow cytometry)

- Transient cyclophosphamide-associated T-regulatory cell depletion
- Transient depletion of effector T-cells, B-cells (not shown)



Conclusions

- Pre-operative, peri-lymphatic cytokine-based immunotherapy is feasible and well tolerated in early stage breast cancer
- Common toxicities included transient grade I/II IRX-related injection site reaction, indomethacin-associated nausea/GI upset
- Increase in sTILs and PD-L1 mRNA/protein

Caveats

- Heterogeneous tumor histology, primarily Luminal subtype (69%)
- No control arms to evaluate biopsy or cyclophosphamide effect
- Uncertain clinical relevance of observed effects

Future Directions

- Ongoing characterization of TILSs via multispectral imaging (PerkinElmer)
- Phase II randomized trial to evaluate pCR rate following neoadjuvant pembrolizumab + chemotherapy +/- IRX-2 in stage II-III TNBC

Thank you!

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