Steering tumor-stroma-immune ecosystem evolution for immune-modulated breast cancer control
## Breast cancer epidemiology

### Female New Cases 2017

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ACS; SEER
Breast cancer epidemiology

2017 Estimated New Cases

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Patient pathway

Stage / Grade
Tumor Node Metastases
Oncotype

Surgery
Chemotherapy
Radiotherapy

ACS; SEER
Breast cancer epidemiology

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### Patient pathway

- **Stage / Grade**
- **Tumor Node Metastases**
- **Oncotype**

- Surgery
- Chemotherapy
- Radiotherapy

ACS; SEER

Stage IIB - IIIC
Fibrosis is an understudied risk factor in breast cancer, and unexplored in the context of anti-tumor immunity.
Fibrosis is an understudied risk factor in breast cancer, and unexplored in the context of anti-tumor immunity.
**Proposal**

**ODE Model**

**Agent Based Models**

**Statistical Analysis**

TCGA / TCC

- **Stroma\text{\textsuperscript{HIGH}} (> 50\%)**
- **Stroma\text{\textsuperscript{LOW}} (< 50\%)**

$p=0.023$

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**Experimental data**

- CD4 CD8/CD8
- CD8 CD8/CD8
TCGA, Breast Cancer, female; Stage IIB – IIIC; neoadjuvant chemotherapy N=501

Stroma\textsuperscript{HIGH} (≥ 50%)
Stroma\textsuperscript{LOW} (≤ 50%)

\[ \text{p}=0.023 \]

Overall Survival

Years since therapy

TIL\textsuperscript{HIGH} (> 0.99%)
TIL\textsuperscript{LOW} (≤ 0.99%)

\[ \text{p}=0.022 \]

Overall Survival

Years since therapy
TCGA, Breast Cancer, female; Stage IIB – IIIC; neoadjuvant chemotherapy N=501

- **Stroma**
  - **HIGH**: (> 50%)
  - **LOW**: (≤ 50%)

- **TIL**
  - **HIGH**: (> 0.99%)
  - **LOW**: (≤ 0.99%)

Overall Survival

Years since therapy

- **p=0.023**
Gene Expression Analysis

- Select genes
- Split patients: median
- Relapse-free survival
- Analyze subsets (N)
  - ER-/HER2-/PR- (255)
  - HER2+ (252)
  - Adjuvant chemotherapy (594)
  - Neoadjuvant chemotherapy (111)
  - Tp53 mutations (188)
- $p < 0.01$

Busch et al. Molecular Cancer (2017)
10-Year Relapse Free Survival

Expression

- **low**
- **high**

Relapse-free survival

**CCL5**

**COL1A1**

Time since treatment

Neoadjuvant Chemo.

- PDGFA
- CD8

Adjuvant Chemo.

- VEGFA
- CCL5

Tp53 mut.

- ***
- **
- ***
- ***
Gene expressions associated with stromal activation potentially predict patient outcomes

- Guide treatment decision
- Potential targets for novel therapeutics
**Statistical Analysis**

TCGA / TCC

- **Stroma**
  - **Stroma\(^\text{LOW}\)** (< 50%)
  - **Stroma\(^\text{HIGH}\)** (> 50%)

- Overall Survival
  - Years since therapy

**Experimental data**

- Survival distribution function
  - False
  - True

- Overall Survival
  - Years since therapy

- Stroma
  - **HIGH** (> 50%)
  - **LOW** (< 50%)

- \(p = 0.023\)

**Proposal**

**ODE Model**

**Agent Based Models**
Activation versus Homeostatic Proliferation in T-cells

Post-thymic Naïve T-cells:
Low affinity for self-peptide-MHC
High affinity for foreign-peptide-MHC
Bioartificial collagen matrix

- Stimulate with homeostatic cytokines
- Stimulate with TCR activation
Lipophilic Dye Dilution Assay

- Generation 0: 1062
- Generation 1: 450
- Generation 2: 925
- Generation 3: 1571
- Generation 4: 1885
- Generation 5: 1629
- Generation 6: 861
- Generation 7: 284

Percent Divided: 60.18
Proliferation Index: 106.85
IL-2: Day 12

Collagen

0μg/ml  1.25μg/ml  2.5μg/ml  5μg/ml  10μg/ml

100 ng/ml  10 ng/ml

CD8+  CD4+
IL-7: Day 12

Collagen

CD8+

CD4+
IL-15: Day 12

Collagen

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100 ng/ml

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CD3/CD28: Day 4

Collagen

### CD8+

- **0µg/ml**
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### CD4+

- **0µg/ml**
- **1.25µg/ml**
- **2.5µg/ml**
- **5µg/ml**
- **10µg/ml**
ABM rules

• Collagen contact inhibits the cell division
• Cells move by Brownian motion
• Once a cell divides both parent and daughter cell become a generation older
• We track the generation distribution at the end of the simulation
An example simulation
Tweakable parameters

- How often cells move per generation?
- How fast the cells escape after collagen contact.
- Collagen density in the well.
Best fit for 2.5 $\mu g/mL$ collagen
Best fit for entire IL-2 assay
Determine functional response of T cell proliferation ($R$) to collagen density

\[
\frac{dR}{dt} = p(C)R
\]

$C_{\text{LOW}}$  

$C_{\text{HIGH}}$  

Simulation  

Exponential Fit  

Simulation  

Exponential Fit
Model evolution
Final model

Model H2

Model diagram with nodes labeled A, R, T, and Col, with arrows indicating the flow of elements labeled as $\beta_{La}$, $\alpha_{La}$, $\delta_T$, $\beta_T$, $\rho_{La}$, $\rho_{Ln}$, and $\rho_T$. The diagram shows the interactions between these elements.
Δ collagen:
\[ \frac{dC}{dt} = \beta_T T + \beta_A A \]

Δ resting lymphocytes:
\[ \frac{dR}{dt} = \psi (R^* - R) + \rho_R e^{-\eta C} - \alpha_T R \]

Δ active lymphocytes:
\[ \frac{dA}{dt} = \alpha_T R + \rho_A A \cdot f(T) - \delta_A A \]

Δ tumor cells:
\[ \frac{dT}{dt} = \rho_T T - \delta_T A T \]
Homeostasis – no tumor

Col

A

T

R
Tumor growth dynamics

Col

A

T

R
Sensitivity to parameters 1/2
Stroma and lymphocytes in patients

N=1097 breast cancer TCGA patients
Sensitivity to initial conditions

- Tumor cell number after ½ year
- Initial stroma
- Initial lymphocytes

Graphs showing:
- Relationship between time and tumor cell number
- Initial lymphocytes and tumor cell number
- Logarithmic scale for tumor cell number
Treatment simulation

vary initial collagen content → ½ year run-in phase → treatment → assess treatment effect
Radiotherapy simulation

5x5 doses of 2 Gy
Chemotherapy simulation

Docetaxel, given every 3 wk

highest Chemo sensitivity
Central Hypothesis: Treatment response and ultimately outcome may be linked to the patient-specific Tumor-Stroma-Immune Ecosystem (TSIE) prior to and its evolution during therapy.

**AIM 1. Determine treatment-induced shifts in Tumor-Stroma-Immune Ecosystem composition.**

1A) Assess TSIE composition (multiplex immunohistochemistry, gene expression) in retrospective TCC biopsy samples and correlate TSIE composition with treatment outcome (pCR).

1B) Calibrate & validate mathematical model dynamics using TSIE and outcome data.

**AIM 2. Predict optimal treatment for immune activation and tumor control.** Simulate if NCT can be escalated where needed and de-escalated where possible.
Patient impact

- Personalization and stratification
- Add to Oncotype Dx 21 Gene Signature
- Identify new therapeutic targets
- Diffusion Weighted MRI
- Improve OS

Graph showing survival distribution funcional
Overall Survival

Years since therapy

Stroma\textsuperscript{LOW} (< 50%)
Stroma\textsuperscript{HIGH} (> 50%)
p=0.023
Budget

• **Aim 1A**: 50 retrospective TCC samples
  • Cytokine analysis, $7.5K
  • Multiplex IHC, $17.5K
    » 7 markers per panel; $350 per panel

• **Aim 1B**
  • Math Modeling postdoc, $20K

• **Aim 2**
  • 30% FTE

• **AACR presentation**, $2.5K
  – (registration, airfare, lodging)

• **Cancer Research publication**, $2.5K

$50K
Thank You.

IMO
PSOC
Moffitt Cancer Center
Sandy Anderson
Danae Paris

Jai Alai
Evolution of dynamic immune state 1/2
Evolution of dynamic immune state 2/2

Guide the trajectory towards a more favorable state

Vary collagen content and lymphocyte numbers at baseline
This does NOT include eta and Rho_R
This DOES include eta and Rho_R
This DOES include eta and Rho_R
This DOES include eta and Rho_R
Patient Data Analysis TCGA Population
n=1085 **

Survival distribution Stroma-Lymphocyte distribution

P value = 0.006

High Lymphocytes Low
Low Lymphocytes High Stroma