T-STM - Enabling Individualized Immunotherapy

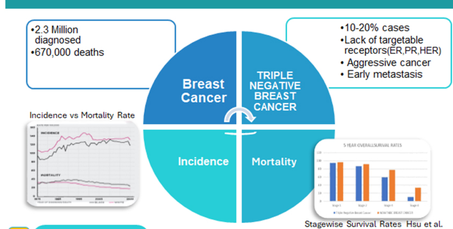
A Spatio-Temporal Modeling of Triple Negative Breast Cancer Tumor-Microenvironment for In-Silico Testing of Multi-Immunosuppressive Inhibitors for Enhanced Immunotherapy

Ajeet S Venkatesh Nikola Tesla Stem High School, Redmond, WA, USA

CBIO054

Introduction

BACKGROUND - CURRENT TREATMENT AND NEED

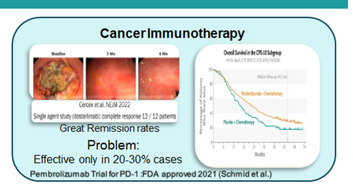


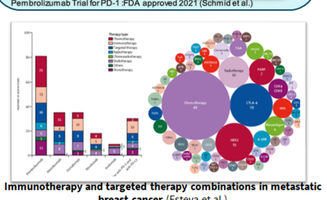
Limitations in Current Therapies

Complex Tumor-microenvironment
- Current drug testing methods **unable to account** for Dynamic interaction of cancer ,immune cells, signaling molecules, extracellular matrix components.
- No mechanism to account for Patient specific variability

Heterogeneity in Tumor-microenvironment
- **Cellular Heterogeneity**: genetics ,epigenetics ,phenotypic variations → growth rates, invasiveness, metastatic potential
- **Molecular Heterogeneity**: Mutations lead to diverse molecular profiles
- **Functional Heterogeneity**: Different metabolic pathways, Spatial heterogeneity due to nutrient availability

Dynamic Immune evasion
- **Multiple Immunosuppressive** pathways and proteins in TME
- Down-regulation of targeted checkpoints or antigen presentation and
- Up-regulation of alternative checkpoints



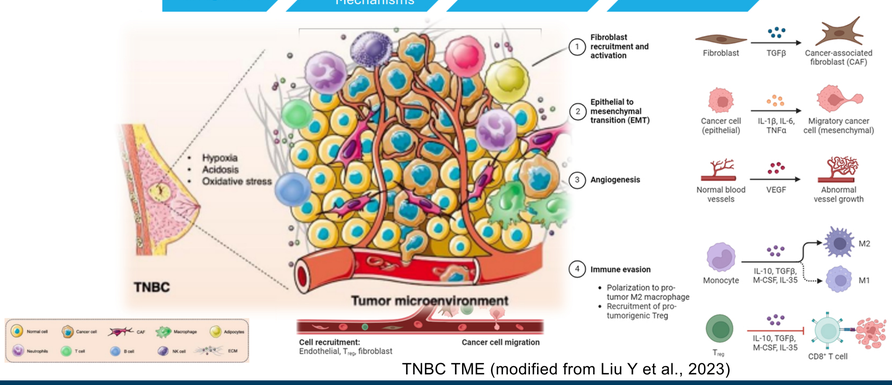


Immunotherapy and targeted therapy combinations in metastatic breast cancer (Esteva et al.)

NEED : Efficient mechanism to simulate the complex cell interactions in the Tumor microenvironment for effective individualized immunomodulatory combination therapy targets identification

CHALLENGE - TUMOR MICROENVIRONMENT HETROGENEITY

Non-Patient Specific Investigations → Dynamic Changes in Immune Mechanisms → Complex TME → Heterogeneity of TME



TNBC TME (modified from Liu Y et al., 2023)

RESEARCH QUESTION & GOALS

Research Question

Can the behavior and interactions of tumor and immune cells in the TNBC TME be modeled spatially and temporally to simulate immune response on the tumor cells and identify potential immunosuppressive targets ?

G1: DEVELOP AGENT MODELS	GOAL 1) Develop agent-based models for Tumor cells, Immune cells ,Stromal cells/Fibroblasts in T-STM
G2: MODEL NUTRIENT DIFFUSSION	GOAL 2) Simulate nutrients diffusion from blood vessels in T-STM using Partial differential equations
G3: MODEL IMMUNO-SUPPRESSIVE PATHWAYS	GOAL 3) Model immunosuppressive mechanims of Kynurenine, AKT mTor and Jak/STAT pathway in T-STM
G4 : SPATIO TEMPORAL VISUALIZATION OF CELLS IN TME	GOAL 4) Develop gifs to visualize Spatio-temporal visuals of tumor, stromal and immune cells to understand probabilistic behavior
G5: TEMPORAL PLOTS	GOAL 5) Develop time plots of tumor, stromal and immune cell counts for analysis and validation
G6 : INHIBITOR IDENTIFICATION	GOAL 6) Identify combination of inhibitors for tumor cell reduction of various molecular subtypes of TNBC

Methodology

Engineering Goal 1,2,3

AGENT MODEL DESIGN

Cell division: Duplication probability of a cell =

$$p_{dupl}\left(i,j\right) = 1 - \exp\left[-\left(\frac{N(i,j)}{\frac{\Theta_{dupl}}{1+\#\,neighbsCAFs(i,j)}\cdot s_{dupl_{stroma}}}\right)^2\right]$$

Where N(i,j) = nutrient concentration in (i,j)
Θ_{dupl} = mutational rate parameter (range set from TCGA BRCA data)

Cell death: Tumor cell death probability=

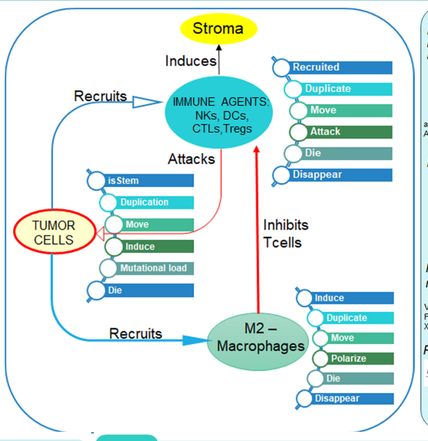
$$p_{necr}\left(i,j\right) = \exp\left[-\left(\frac{M(i,j)}{\theta_{necr}}\right)^2\right]$$

Where M(i,j) = nutrient concentration for cell in (i,j)
Θ_{necr} = necrosis parameter (calculated from mutational status)

Cell movement : Probability =

$$p_{move}\left(i,j\right) = 1 - \exp\left[-\left(\frac{M(i,j)}{\frac{\theta_{move}}{1+\#\,neighbsCAFs(i,j)}}\right)^2\right].$$

Θ_{move} = parameter set for cell movement in Moore's neighborhood
Dependent on CAF diffusion, stromal permeability
Where M(i,j) = nutrient concentration in (i,j)



Recruitment :
Probability of recruitment of immune cells proportional to adjuvanticity signal in Moore's neighborhood

$$p_{recruit}(i,j) = \exp\left[-\left(\frac{1}{\text{adjuvanticity}_{neigh}(i,j)}\right)\right]$$

adjuvanticity$_{neigh}$ = summation of adjuvant signals in Moore neighborhood
Adjuvanticity signal ↑ cytokines, tumor cell death, ↓ immunosuppressants

Recruitment of Immunosuppressive agents:

$$p_{TREG_{recruit}}(i,j) = \exp\left[-\left(\frac{\text{inhib\_TREG\_recruit\_par}}{\text{inhibitory}_{neigh}\ (i,j)}\right)\right].$$

$$p_{CAF_{recruit}}\left(i,j\right) = \exp\left[-\left(\frac{\text{inhib\_CAF\_recruit\_par}}{\text{inhibitory}_{neigh}\ (i,j)}\right)\right].$$

Rate of production of immune suppressive metabolites

$$\frac{dx_i}{dt} = fi\,(x(t)P)$$

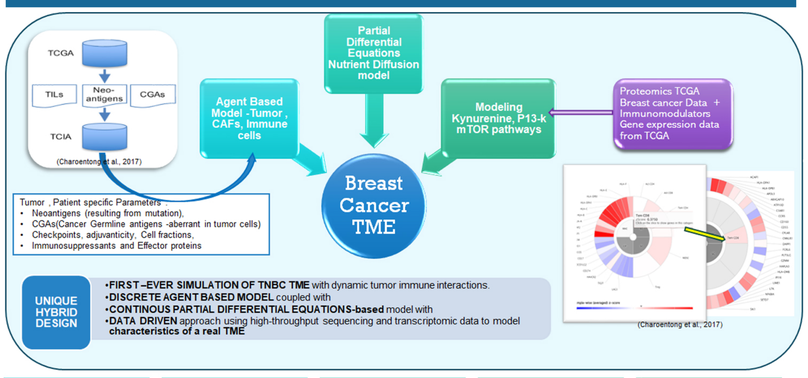
Varies per metabolite
Fi =nonlinear function representing number of metabolites (x1,x2..x3..x4)
X=metabolite transformation : m1/m2

PDE Representing Nutrient Diffusion

$$\frac{\partial C(x,y,t)}{\partial t} = D_c\nabla^2 C(x,y,t) - k_i\,I(x,y,t)\,C(x,y,t) - k_i\,T(x,y,t)\,C(x,y,t)$$

Where D_c is the nutrient diffusion coefficient, I(x,y,t) and T(x,y,t) are the spatial distributions at time t of IS and cancer – related agents, respectively, and k_i and k_i are degradation coefficients

T-STM MODEL DESIGN

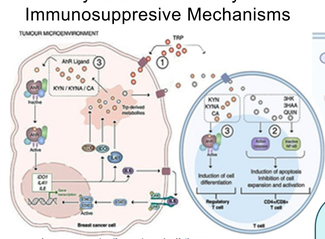


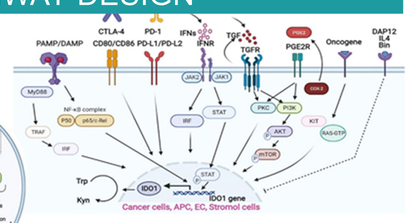
UNIQUE HYBRID DESIGN
- **FIRST –EVER SIMULATION OF TNBC TME** with dynamic tumor immune interactions.
- **DISCRETE AGENT BASED MODEL** coupled with
- **CONTINOUS PARTIAL DIFFERENTIAL EQUATIONS-based** model with
- **DATA DRIVEN** approach using high-throughput sequencing and transcriptomic data to model **characteristics of a real TME**

UNIQUE HYBRID DESIGN → AGENT BASED MODELING → IMMUNO-MODULATION → NUTRIENT MODELING → IMMUNO-SUPPRESSIVE PATHWAYS

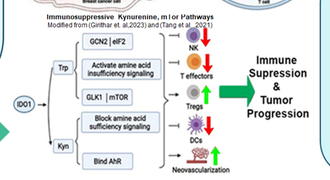
NUTRIENT DIFFUSION & IMMUNOSUPRESIVE PATHWAY DESIGN

Kynurenine Pathway Immunosuppresive Mechanisms





Immunosuppressive Kynurenine, m i or Pathways
Modified from (Gnthar et al.,2023) and (Tang et al., 2021)



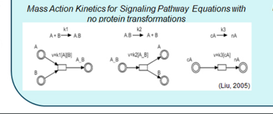
Nutrient Diffusion using Partial differential equations:

$$\frac{\partial C(x,y,t)}{\partial t} = D_c\nabla^2 C(x,y,t) - k_i\,I(x,y,t)\,C(x,y,t) - k_i\,T(x,y,t)\,C(x,y,t),$$

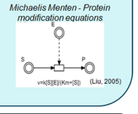
Where D_c is the nutrient diffusion coefficient , $I(x,y,t)$ and $T(x,y,t)$ *are the spatial distributions of Immune agents and Tumor agents at time t and k_i and K_i are respective consumption rates.*

Biological Pathways Modeling

Mass Action Kinetics for Signaling Pathway Equations with no protein transformations



Michaelis Menten - Protein modification equations



2 Nutrient types modeling :N- Essential for cell division; M-Essential for cell survival ;
Modeled 51 differential equations for the immunosuppressive pathways

CANCER AGENTS
- Accumulate mutations resulting :
- 1)loss or acquisition of antigenicity or new antigens
- (2) loss of immunogenicity through inhibitory immune checkpoint molecules(LAG3, PDL1,TIGIT etc.)
- (3) release immunosuppressive signaling molecules & increased or decreased duplication/survival

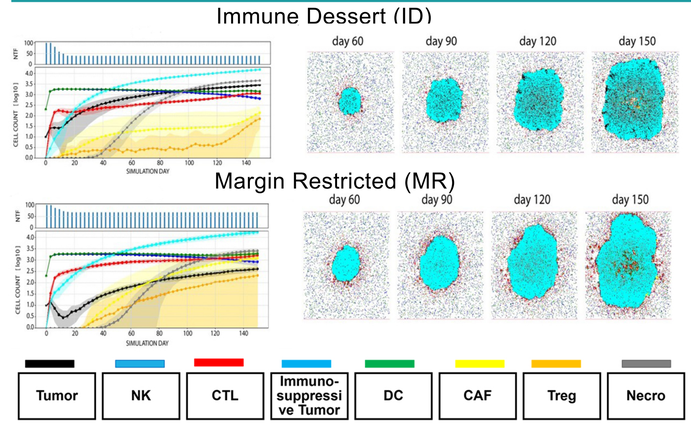
CAFS
- Efferent mechanism: cancer cells trigger a response in the stroma,
- Afferent mechanism : Activated CAFs affect proliferation
- Parameter degree of permeability- TILs infiltration, tumor cell movement

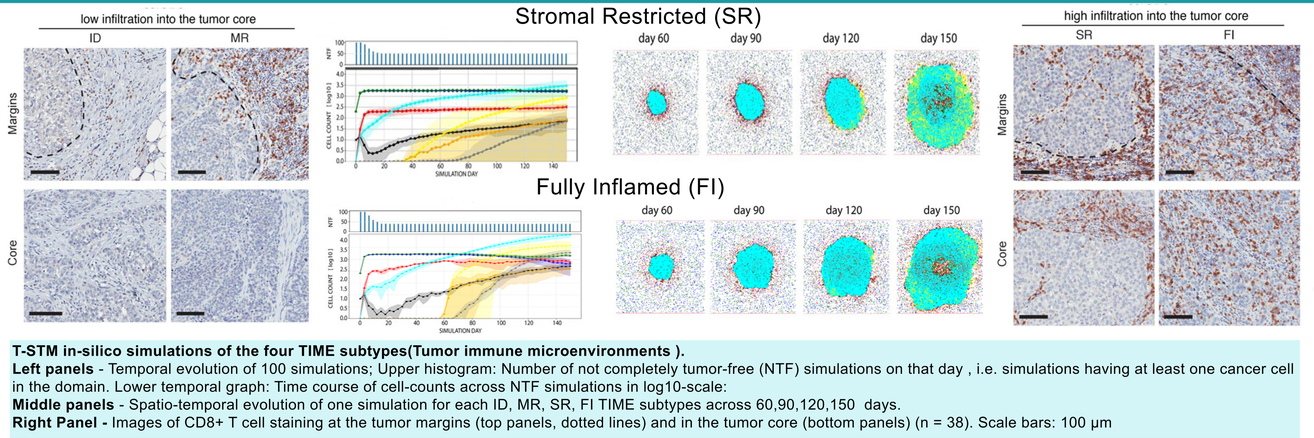
IMMUNE AGENTS
- NKs, CTLs (CD4, CD8), Dendritic Cells(DCs) and immunosuppressive Tregs
- Macrophages polarize from Immune effector M1 to immune suppressive M2
- Treg recruitment : when a CTL becomes inactive or immunosuppressive pathway

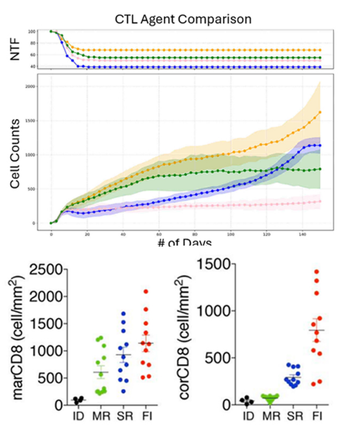
CTLS
- Adjuvanticity parameter : Increases with chemokines , decreases with Tregs /Metabol
- CTLs killing probability lowered when tumor acquires checkpoint mutation -PDL1/ LAG3
- Antigen specificity of CTLs: CTLs attack only when specific antigens are recognized .
- Antigen-specific T cells recruited if recognized or delayed, so to mimic their activation in the lymph nodes.

Results and Data Analysis

T-STM VALIDATION: HISTOPATHOLOGICAL CORRELATION WITH 4 IMMUNE SUBTYPES OF TNBC TME







T-STM in-silico simulations of the four TIME subtypes(Tumor immune microenvironments).
Left panels - Temporal evolution of 100 simulations; Upper histogram: Number of not completely tumor-free (NTF) simulations on that day , i.e. simulations having at least one cancer cell in the domain. Lower temporal graph: Time course of cell-counts across NTF simulations in log10-scale:
Middle panels - Spatio-temporal evolution of one simulation for each ID, MR, SR, FI TIME subtypes across 60,90,120,150 days.
Right Panel - Images of CD8+ T cell staining at the tumor margins (top panels, dotted lines) and in the tumor core (bottom panels) (n = 38). Scale bars: 100 μm

Cardinality of CTL cells over time for ID,MR,SR and FI . Upper subgraph : number of NTF (non-tumor free) simulations
Lower subgraph: Avg # of CTLs computed across all NTF simulations of given TIME group shaded area : (± standard deviation)

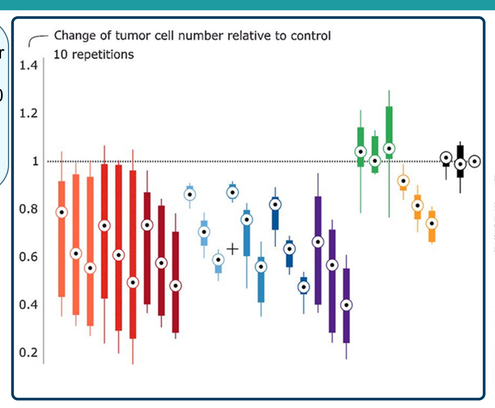
Quantification of CD8+ T cell densities at the tumor margins (marCD8) and in the tumor core (corCD8) (n = 38) from (Gruosso et al., 2019).

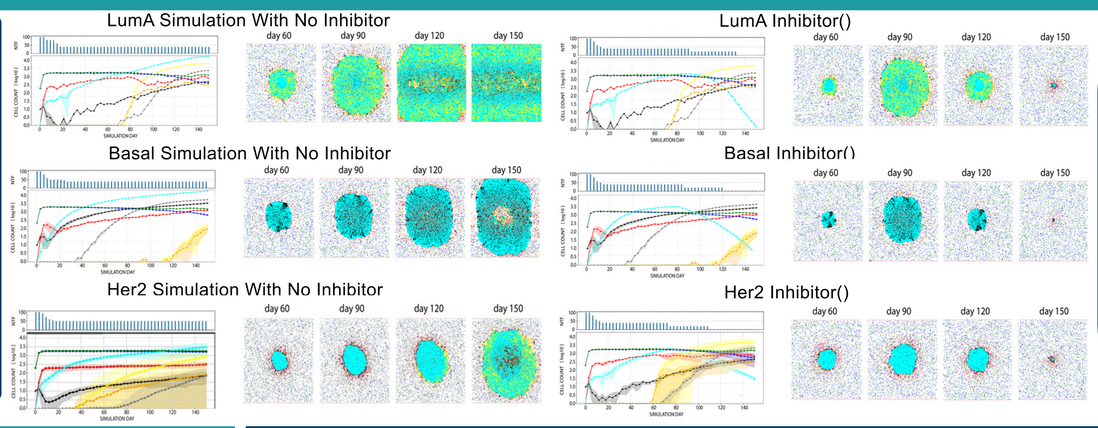
Clinical Significance :
T-STM ran simulations on 38 datasets and successfully reproduced the 4 Tumor microenvironment immune phenotypes of the TNBC TME - Immune desert(ID), Margin restricted (MR),Stromal restricted (SR) and Fully inflamed(FI), validating an in-silico simulation platform that can be used for inhibitor tests. Immune phenotype classification based on CD8+ T cell localization and histopathological slides derived from (Gruosso et al., 2019)

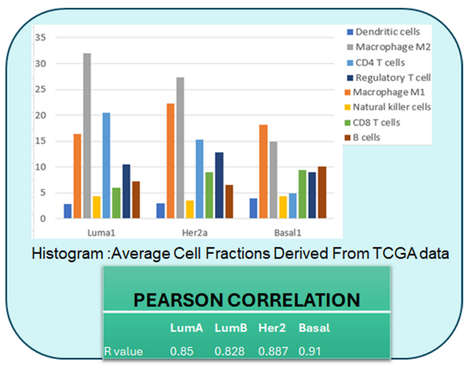
T-STM IN-SILICO TESTING: INHIBITOR COMBINATIONS FOR TNBC MOLECULAR SUBTYPES

Simulation Details:
Input : TCGA TNBC patient data (n=30) for each of molecular subtypes –Basal like, Luminal Androgen receptor, Mesenchymal, immunomodulatory molecular subtype Ran 30 simulations of each inhibitor individually and in combination for 150 days or until tumor free for each dataset
Best Representative simulation results plot shown below for each molecular subtype

- Lum A : Tlr9 (Tol like receptor 9 agonist) immunostimulant + AR inhibitor + PDL1 blockade
- Basal : PDL1+LAG3+ IDO1 inhibitor +p13K inhibitor
- HER2 : Tlr9 (Tol like receptor 9 agonist) immunostimulant + mTOR inhibitor + LAG3 blockade + PDL1 blockade
- Lum A: PDL1 inhibitors + Kynu inhibitor
- Her2: IDO1 inhibitor + PDL1 inhibitor
- Immunomodulatory: PDL1 + LAG 3 blockade + IDO1 inhibitor
- Lum B : LAG3 blockade +IDO1 inhibitor+ PDL1 blockade + p13K inhibitor
- Basal 2: IDO1 + LAG3 inhibitor
- Basal 1: IDO1 + PDL1 blockade
- Control
- Median







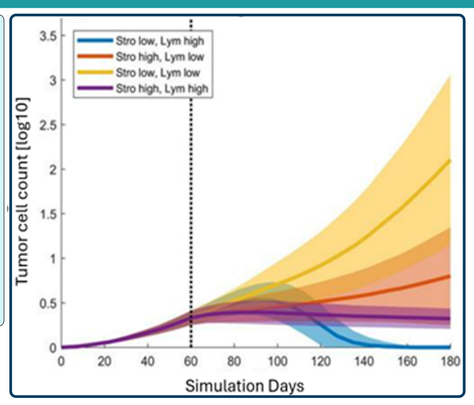
Histogram :Average Cell Fractions Derived From TCGA data

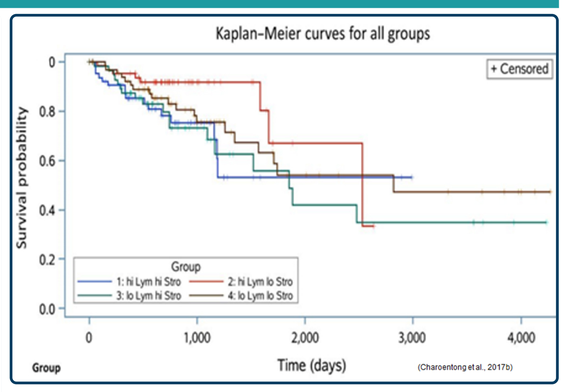
PEARSON CORRELATION

	LumA	LumB	Her2	Basal
R value	0.85	0.828	0.887	0.91

T-STM SIMULATION : EFFECT OF STROMAL AND T-LYMPHOCYTE RATIOS ON TUMOR ERADICATION

Simulation Procedure
Input : TCGA TNBC basal data (n=50)
50 simulations on each dataset
1) Grow tumor for 60 days
2) On day 60, simulated combinations: varying levels of stroma & CAF recruitment vs CTLS (T lymphocytes).
3)Recorded Tumor cell counts





Findings :
Stro low, T-Lym low : Unhindered, exponential tumor growth
Stro high, T-Lym low : Restrained tumor growth, as expected
Stro high, T-Lym high : Tumor size constrained to <10,000 cells
Stro low, T-Lym high : Initial increase in tumor mass, then immune cells inflow completely eradicated tumor in almost all simulation runs.

Simulation results correlate with Kaplan–Meier survival curves for all groups and Cox proportional hazards model (hazard ratio 0.309 for overall survival 0.322, P = 0.0219, \confidence interval 0.122 to 0.849)

Clinical Significance :
Significant finding - shows that stromal permeability can play a key role in immune response and CTL infiltration. Hence Stroma targeting therapies that increase stromal permeability optimally can be given to ensure increased immune infiltration and drug delivery for effective combination immunotherapy.

CONCLUSIONS

T-STM - **novel mechanism** for **inidivualized custom immunotherapy by simulating tumor microenvironment dynamics** for
- In-silico testing potential **individualized combination therapies** based on the patient's transcriptomic/proteomic data rather than generalized molecular subtype-based therapy.
- First step in customized optimal combination immunotherapy
- Potential to save decades in drug discovery and billions of dollars in clinical trials

Successful Validation
- T-STM spatio-temporal gifs showed accurate TCGA histopathological immune phenotypes reproduction
- High correlation with cell counts ratios from T-STM simulations for each of the 4 TNBC molecular subtypes with the cell fraction histograms independently derived from TCGA datasets

Identified **novel combination inhibitors**
- Identified best inhibitor combinations for basal ,immunomodulatory, Her2 and Lum A TNBC datasets
  - Blockades for LAG3 and PDL1 checkpoints along with IDO1 and p13K inhibitors
  - The Tlr9 agonist showing results in Lum A and Her2 makes sense as they have higher number of polarized immunosuppressive macrophages and TLR9 agonist repolarizes from M2 -> M1

Significant finding: **Stromal permeability can play a key role in immune response and CTL infiltration.**
- Stroma targeting therapies that increase stromal permeability optimally can be given to ensure increased immune infiltration and drug delivery for effective combination immunotherapy.

FUTURE POTENTIAL
T-STM : can be further developed as a platform to test out potential individualized custom therapies based on patient data utilizing predictive analytics for safety tests and used in the discovery of novel therapeutics as well.
Next steps :
- Enhance simulation by modeling other immunomodulatory pathways and potential microbiome interactions
- AI model interacts with simulation to calculate potential drug concentrations based on simulated remission rates
- Run wet lab tests on tumor cell lines to validate the findings of combination therapies.
- Model molecularly imprinted polymers with high binding affinity in place of antibodies for cost effective custom multi drug targets potential
- Develop method of collecting temporal transcriptomic data to act as further vallidation/verification
- Use temporal data with AI discriminator to further fine tune proteonomic interactions and cellular behavior
- Fine tune patient specific combination therapy concentrations to minimize immunoinflamation/ toxicity

LIMITATIONS
- T-STM modelled only 2 immunosuppressive pathways. It can be used for predictive analytics when all major immunomodulatory pathways along with gene transcription have been modeled. The model didn't include monocytes and other effector cells