Team Yellow
IMO Workshop 7

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

Sarah Susan Audrey Hatem Renee

Heiko

Adam

Enakshi

Jakob

Daniel

Inom

Tom

MOFFITT CANCER CENTER

# Breast cancer epidemiology

2017 Estimated New Cases

| Female | | |
|---|---|---|
| Breast | 252,710 | 30% |
| Lung & bronchus | 105,510 | 12% |
| Colon & rectum | 64,010 | 8% |
| Uterine corpus | 61,380 | 7% |
| Thyroid | 42,470 | 5% |
| Melanoma of the skin | 34,940 | 4% |
| Non-Hodgkin lymphoma | 32,160 | 4% |
| Leukemia | 25,840 | 3% |
| Pancreas | 25,700 | 3% |
| Kidney & renal pelvis | 23,380 | 3% |
| **All sites** | **852,630** | **100%** |

2017 Estimated Deaths

| Female | | |
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| Lung & bronchus | 71,280 | 25% |
| Breast | 40,610 | 14% |
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| Pancreas | 20,790 | 7% |
| Ovary | 14,080 | 5% |
| Uterine corpus | 10,920 | 4% |
| Leukemia | 10,200 | 4% |
| Liver & intrahepatic bile duct | 9,310 | 3% |
| Non-Hodgkin lymphoma | 8,690 | 3% |
| Brain & other nervous system | 7,080 | 3% |
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*ACS; SEER*

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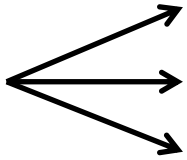
# Patient pathway

Stage / Grade
Tumor Node Metastases
Oncotype



Surgery
x
Chemotherapy
x
Radiotherapy

# Breast cancer epidemiology

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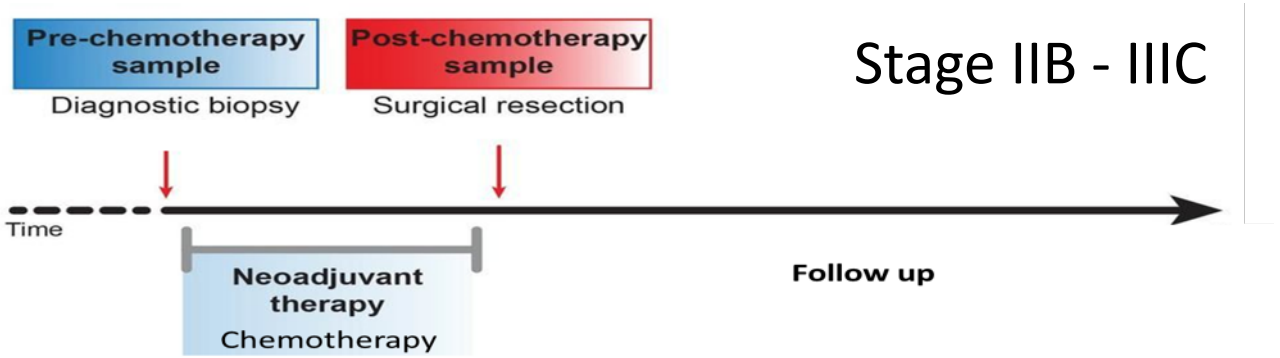
*ACS; SEER*

# Patient pathway

Stage / Grade
Tumor Node Metastases
Oncotype

→ Surgery
x Chemotherapy
x Radiotherapy




Stage IIB - IIIC

# Fibrosis is an understudied risk factor in breast cancer, and unexplored in the context of anti-tumor immunity

Cancer Epidemiology, Biomarkers & Prevention

## The Association of Measured Breast Tissue Characteristics with Mammographic Density and Other Risk Factors for Breast Cancer

Tong Li,[1] Limei Sun,[1] Naomi Miller,[3] Trudey Nicklee,[3] Jennifer Woo,[3] Lee Hulse-Smith,[3] Ming-Sound Tsao,[3] Rama Khokha,[2] Lisa Martin,[1] and Norman Boyd[1]

Divisions of [1]Epidemiology and Statistics and [2]Experimental Therapeutics, Ontario Cancer Institute and [3]Department of Pathology, Princess Margaret Hospital, Toronto, Ontario, Canada

RESEARCH ARTICLE Open Access

## Elevated collagen-I augments tumor progressive signals, intravasation and metastasis of prolactin-induced estrogen receptor alpha positive mammary tumor cells

Craig E. Barcus[1,2], Kathleen A. O'Leary[2], Jennifer L. Brockman[2], Debra E. Rugowski[2], Yuming Liu[5], Nancy Garcia[2], Menggang Yu[4,6], Patricia J. Keely[1,3,5,6], Kevin W. Eliceiri[5,6] and Linda A. Schuler[1,2,6*]

## Is Collagen an Independent Risk Factor for Breast Cancer?

Paola Taroni, Anna Maria Paganoni, Francesca Ieva, Francesca Abbate, Enrico Cassano, Rinaldo Cubeddu, and Antonio Pifferi

Author Information · Find other works by these authors

Clinical and Translational Biophotonics
2016
Fort Lauderdale, Florida United States
25–28 April 2016
ISBN: 978-1-943580-10-1

From the session
Optical Biomarkers II (TTh4B)

REVIEW

## Mammographic density and breast cancer risk: current understanding and future prospects

Norman F Boyd*[1,2], Lisa J Martin[1,2], Martin J Yaffe[3] and Salomon Minkin[2]

PLOS ONE

RESEARCH ARTICLE

## The Value of Tumor Infiltrating Lymphocytes (TILs) for Predicting Response to Neoadjuvant Chemotherapy in Breast Cancer: A Systematic Review and Meta-Analysis

Yan Mao[1], Qing Qu[2], Yuzi Zhang[1], Junjun Liu[1], Xiaosong Chen[1], Kunwei Shen[1]*

1. Comprehensive Breast Health Center, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China, 2. Department of Oncology, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China

BJC
British Journal of Cancer (2014) 111, 157–165 | doi: 10.1038/bjc.2014.279

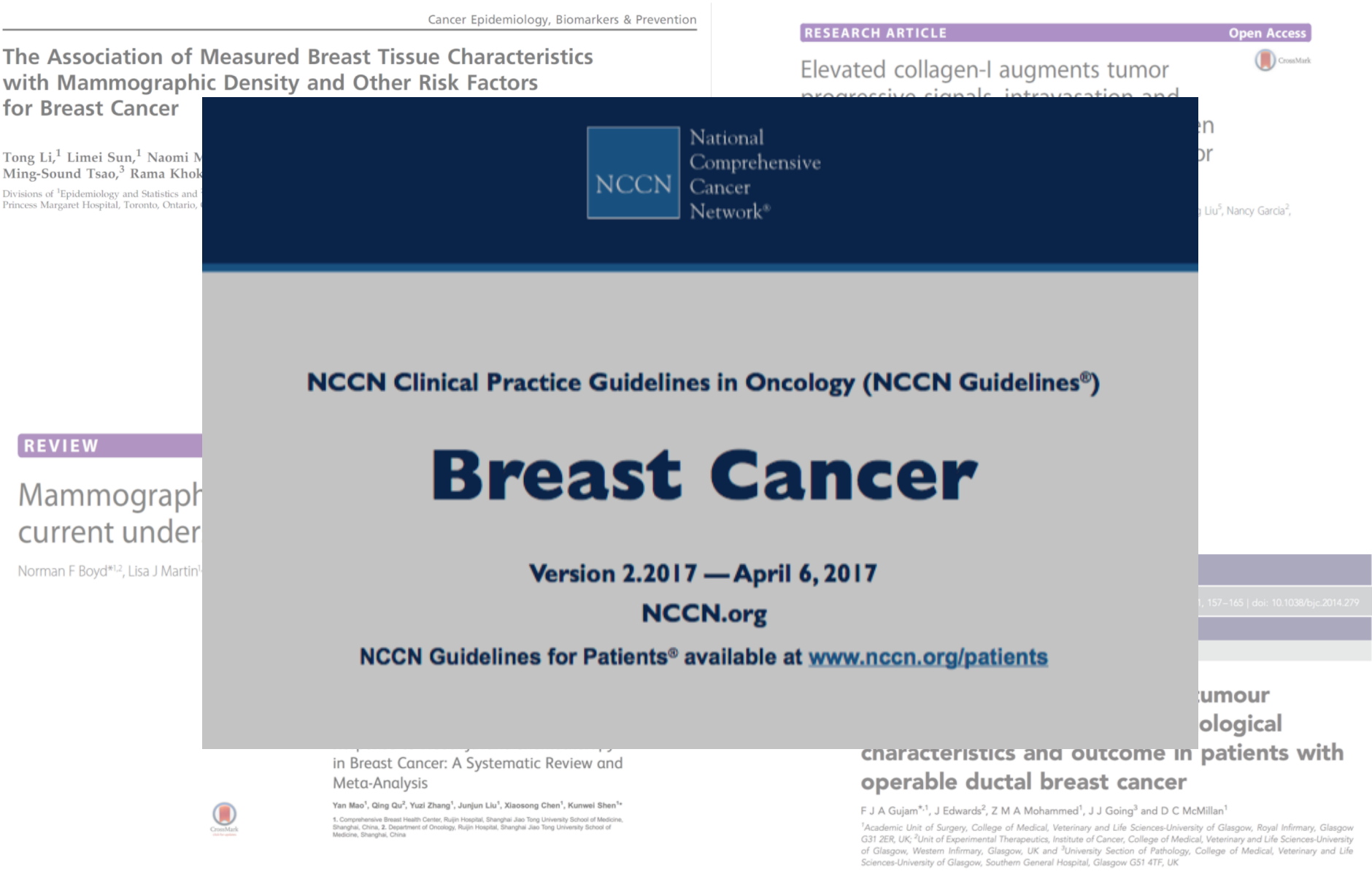
Keywords: tumour stroma; tumour microenvironment; breast cancer; survival

## The relationship between the tumour stroma percentage, clinicopathological characteristics and outcome in patients with operable ductal breast cancer

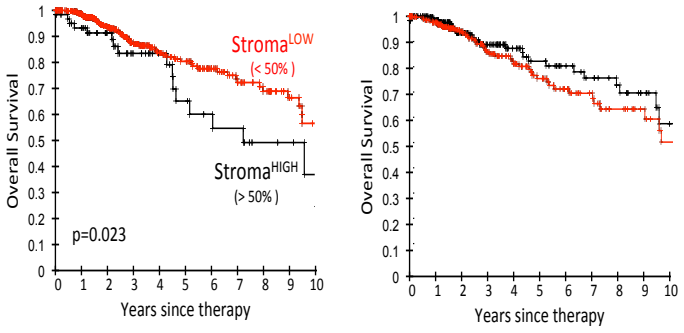
F J A Gujam*[,1], J Edwards[2], Z M A Mohammed[1], J J Going[3] and D C McMillan[1]

[1]Academic Unit of Surgery, College of Medical, Veterinary and Life Sciences-University of Glasgow, Royal Infirmary, Glasgow G31 2ER, UK; [2]Unit of Experimental Therapeutics, Institute of Cancer, College of Medical, Veterinary and Life Sciences-University of Glasgow, Western Infirmary, Glasgow, UK and [3]University Section of Pathology, College of Medical, Veterinary and Life Sciences-University of Glasgow, Southern General Hospital, Glasgow G51 4TF, UK

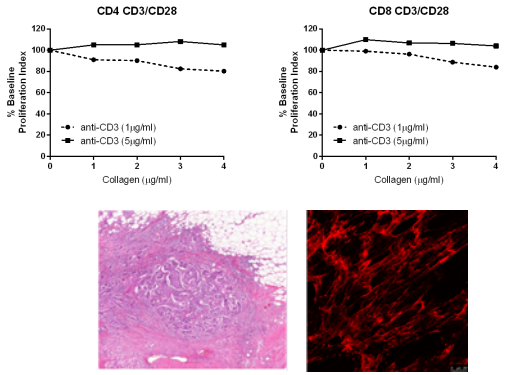
# Fibrosis is an understudied risk factor in breast cancer, and unexplored in the context of anti-tumor immunity



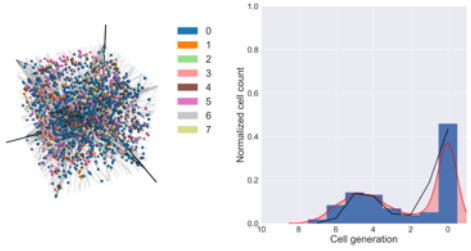
## Statistical Analysis
## TCGA / TCC



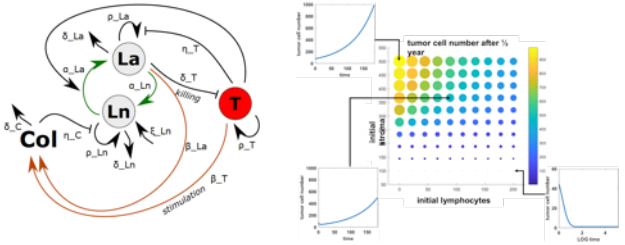

## Experimental data




## Agent Based Models

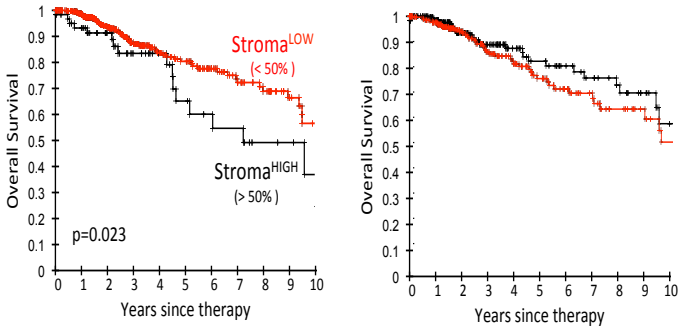



## ODE Model




## Proposal

## Statistical Analysis
## TCGA / TCC

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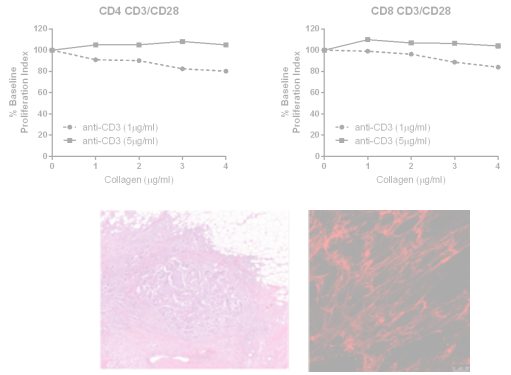


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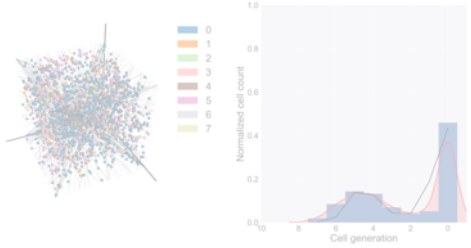


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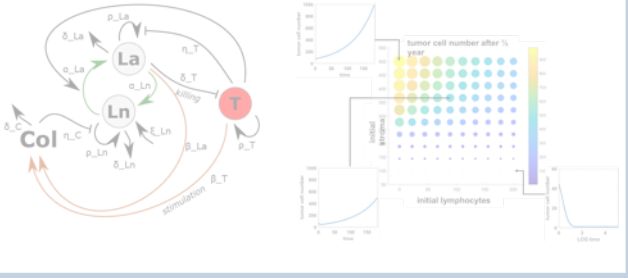
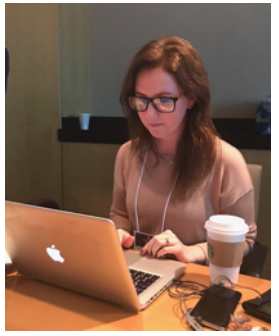
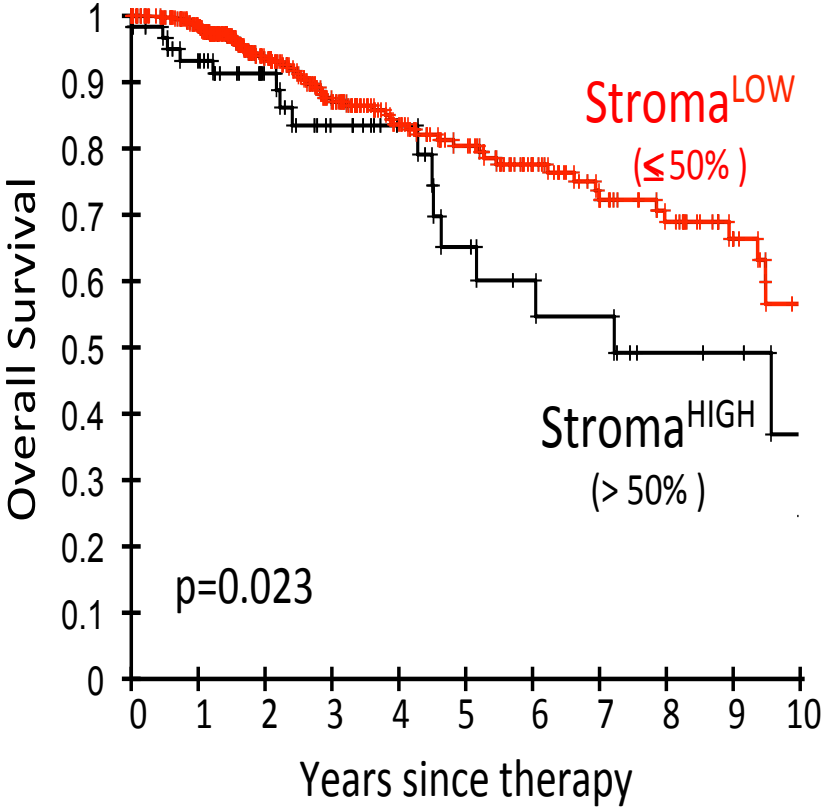
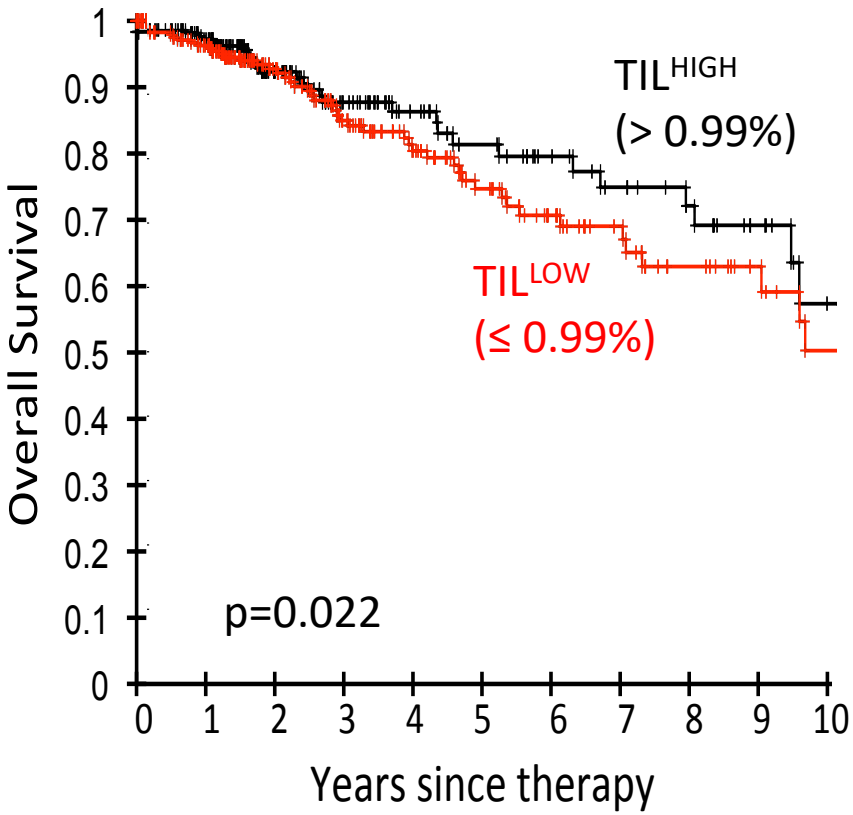



## Proposal

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





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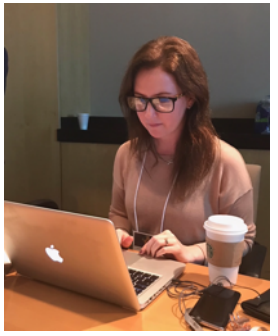
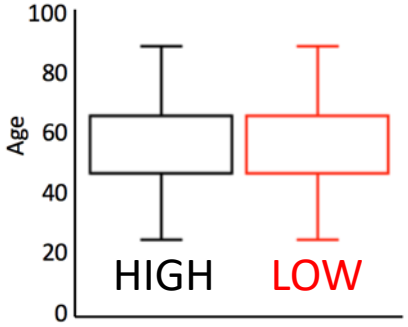
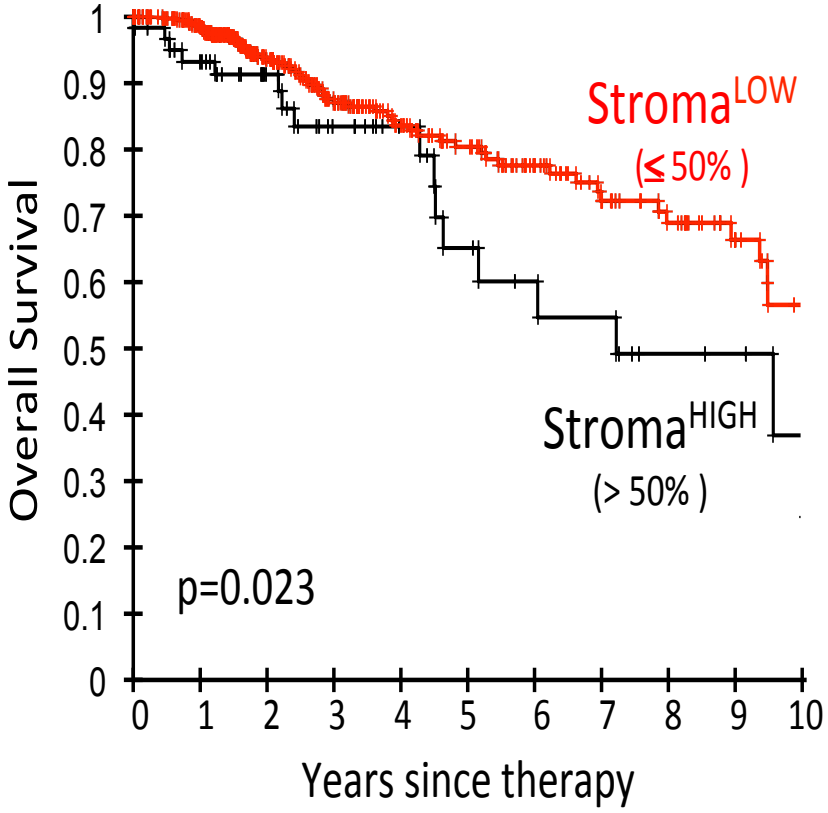
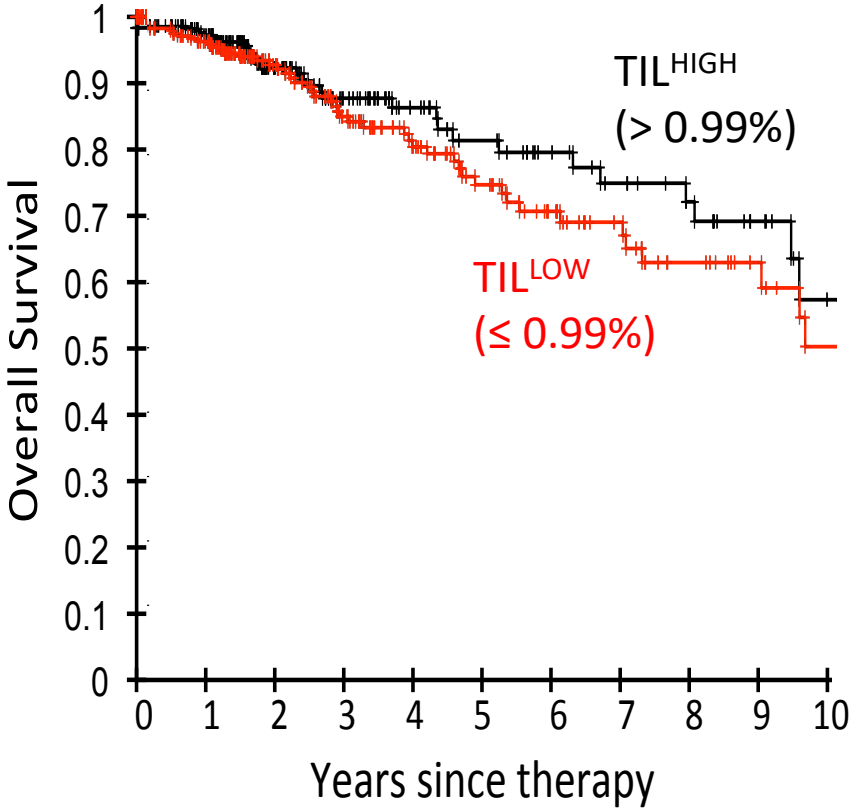




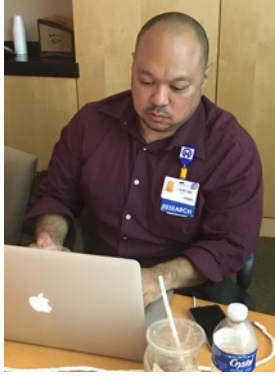



Kaplan-Meier Plotter

kmplot.com

# 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)
- $p < 0.01$

ACTA2 (↑)
ALDH1A3 (↓)
CCL5 (↑)
CXCL12
NANOG (↑)
PDGFA (↑)
SOX2 (↑)
TGFB1 (↑)

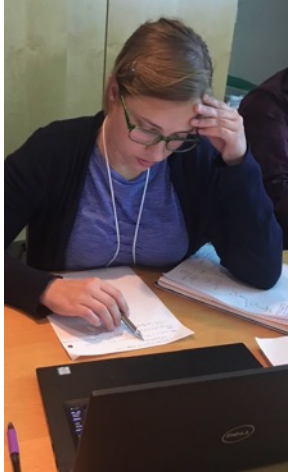
**FIBROBLAST ACTIVATION***

COL1A1 — **Collagen Creation**

CD3
CD8 — **T Cells**

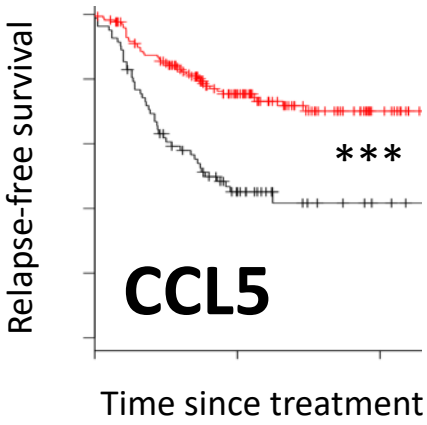
Gyorffy *et al. Breast Cancer Res Treatment* (2010)
Busch *et al. Molecular Cancer* (2017)

# 10-Year Relapse Free Survival

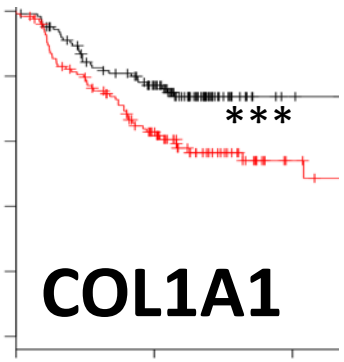


Expression
low
high

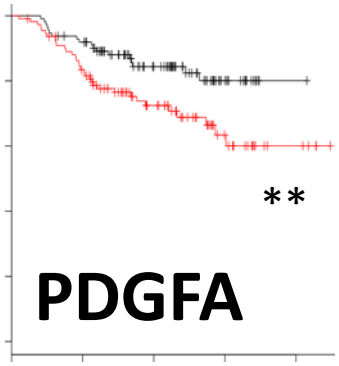
ER-/HER2-/PR-

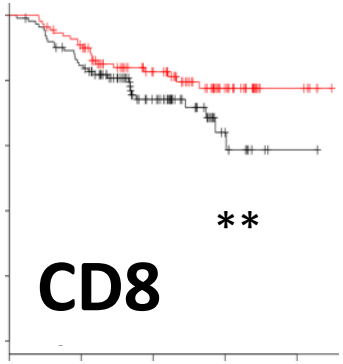



HER2+

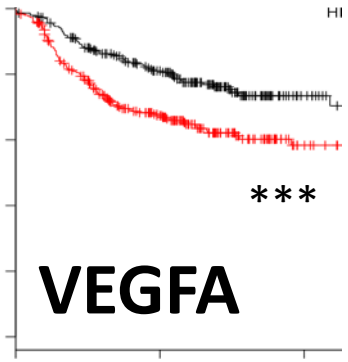



Neoadjuvant Chemo.

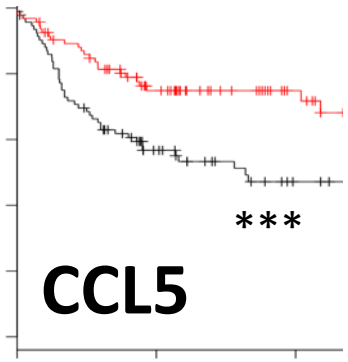


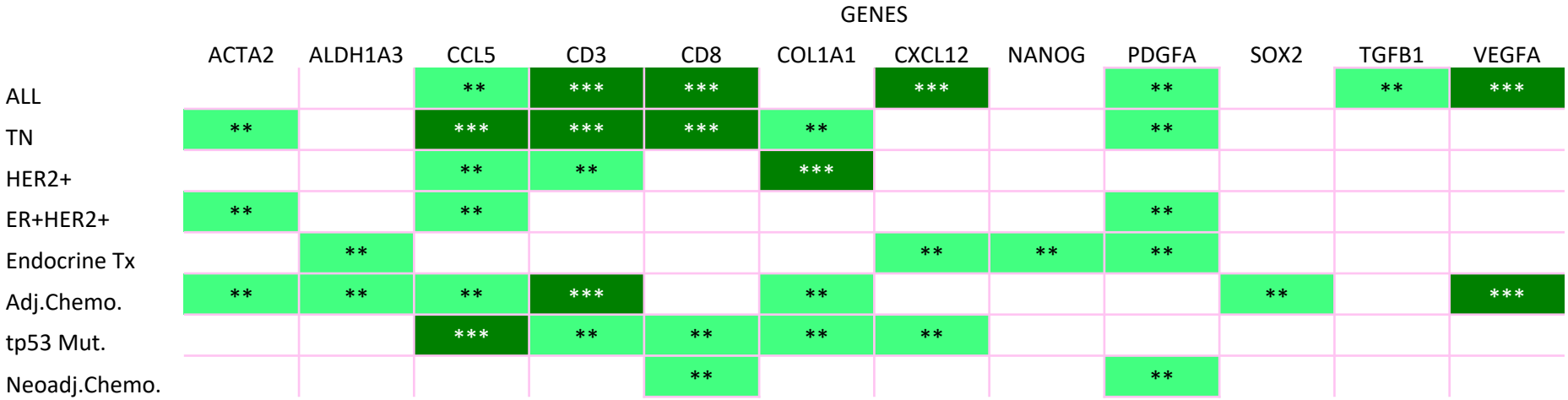

Adjuvant Chemo.




Tp53 mut.



# Gene expressions associated with stromal activation potentially predict patient outcomes




- Guide treatment decision
- Potential targets for novel therapeutics

## Statistical Analysis
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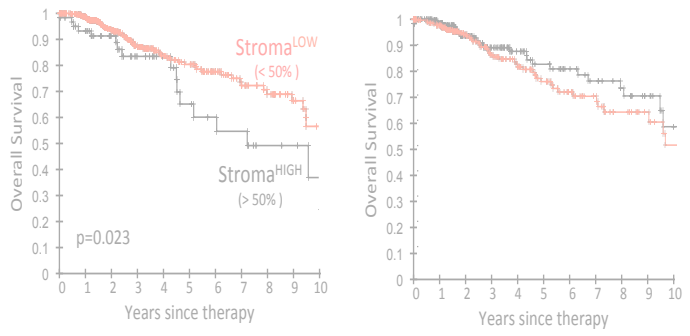


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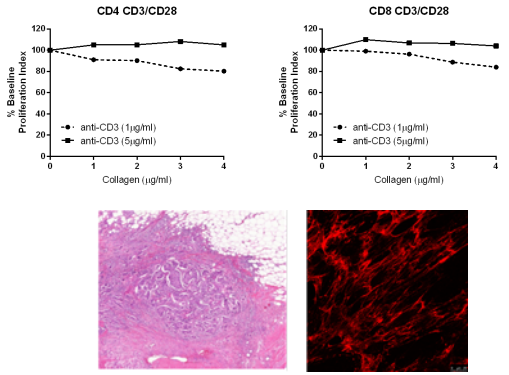
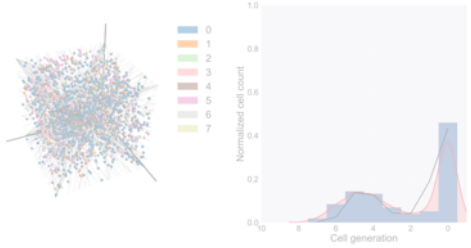
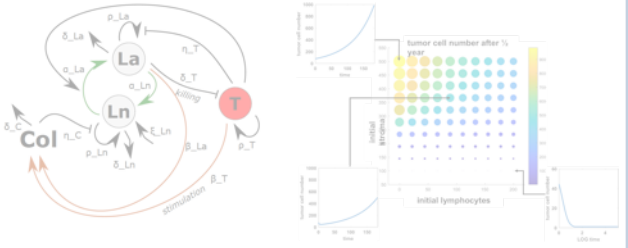



## Agent Based Models



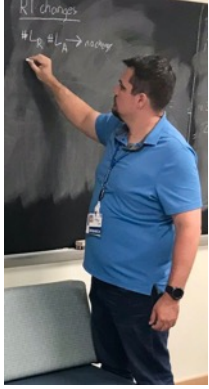

## ODE Model

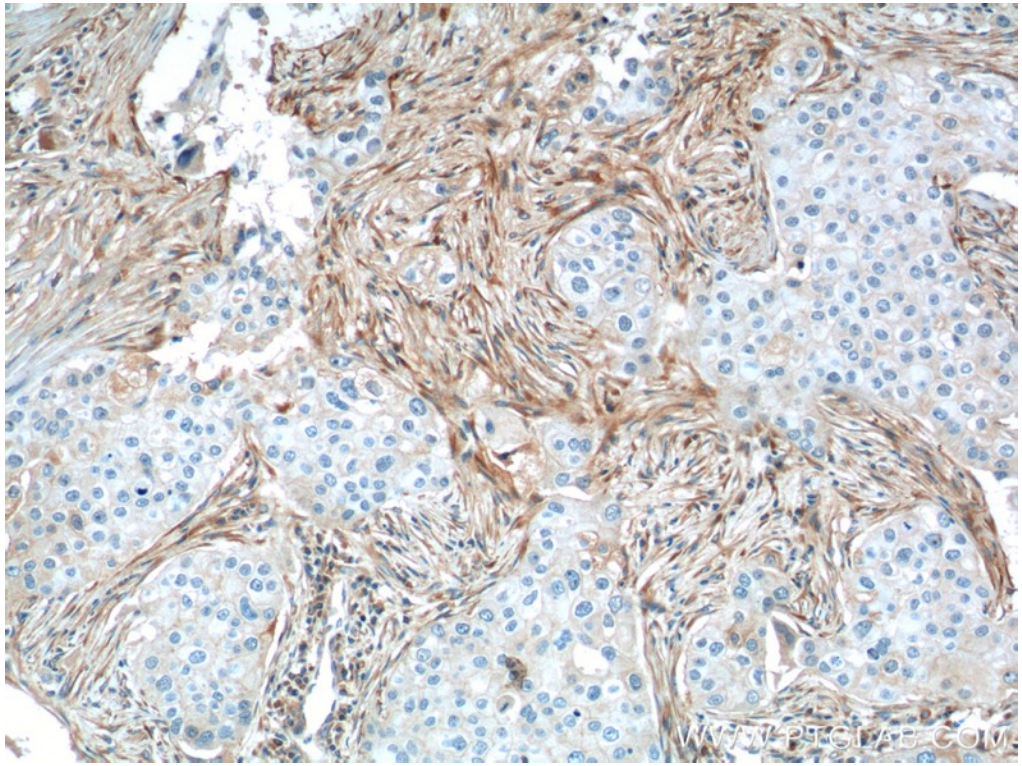



## Proposal

# Activation versus Homeostatic Proliferation in T-cells

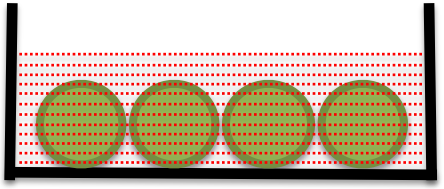
**Post-thymic Naïve T-cells:**
**Low affinity for self-peptide-MHC**
**High affinity for foreign-peptide-MHC**





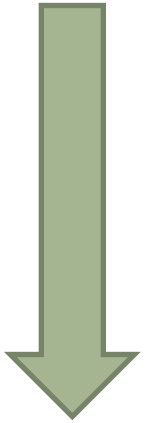
https://www.ptglab.com/Products/Pictures/COL1A2-Antibody-14695-1-AP-IHC-70256.jpg

ProteinTech

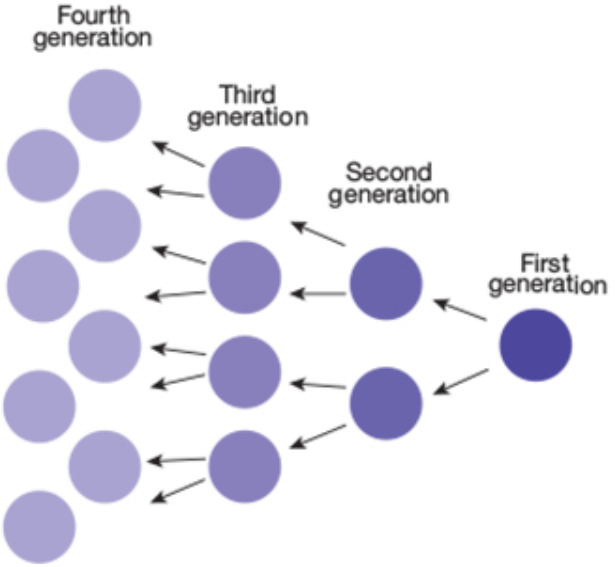


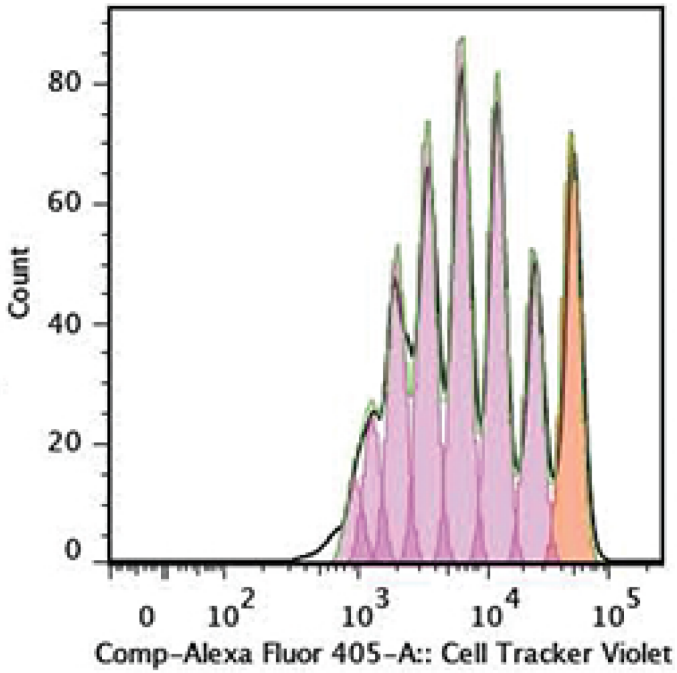
# Bioartificial collagen matrix

- Stimulate with homeostatic cytokines
- Stimulate with TCR activation



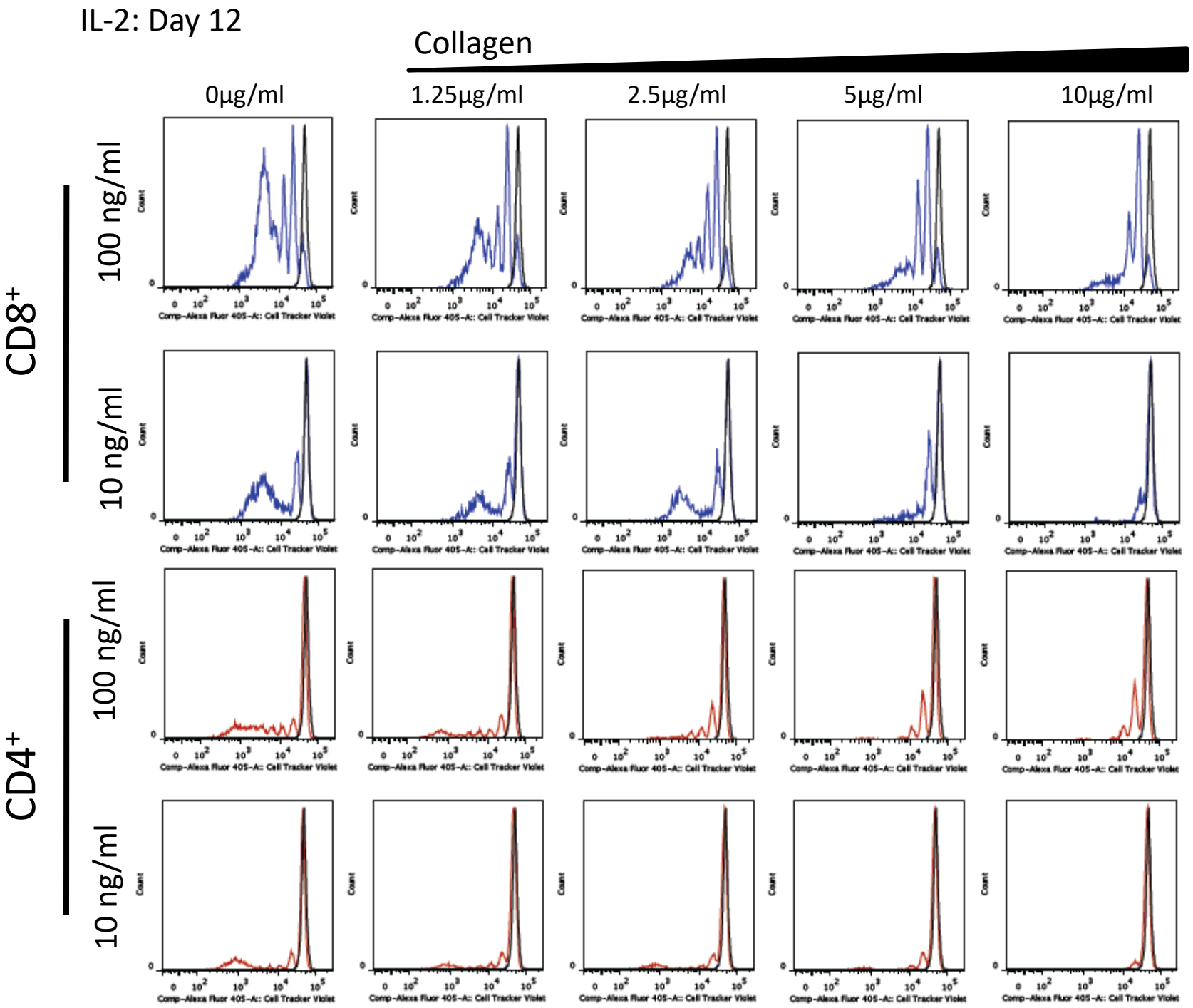
# Lipophilic Dye Dilution Assay

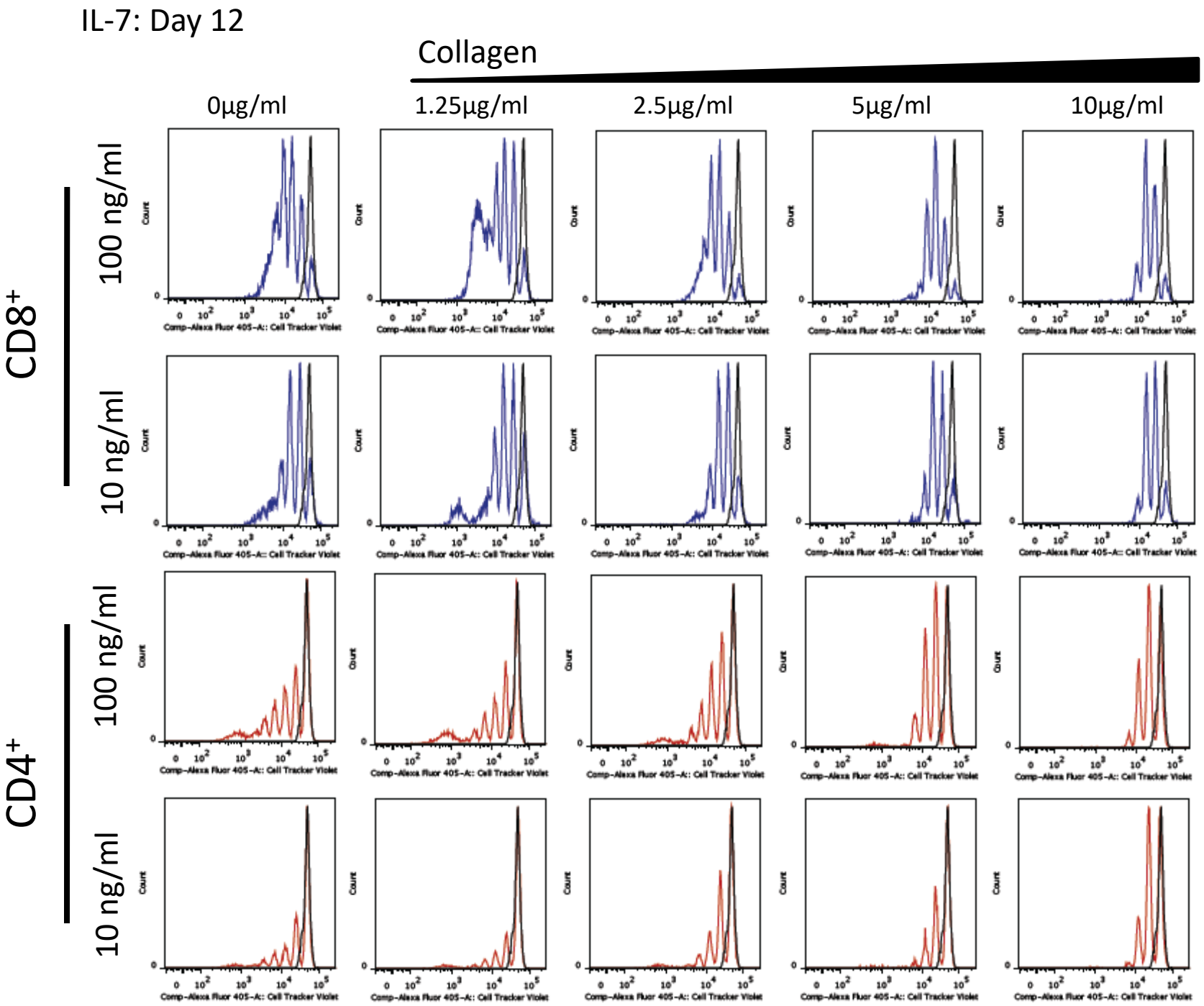


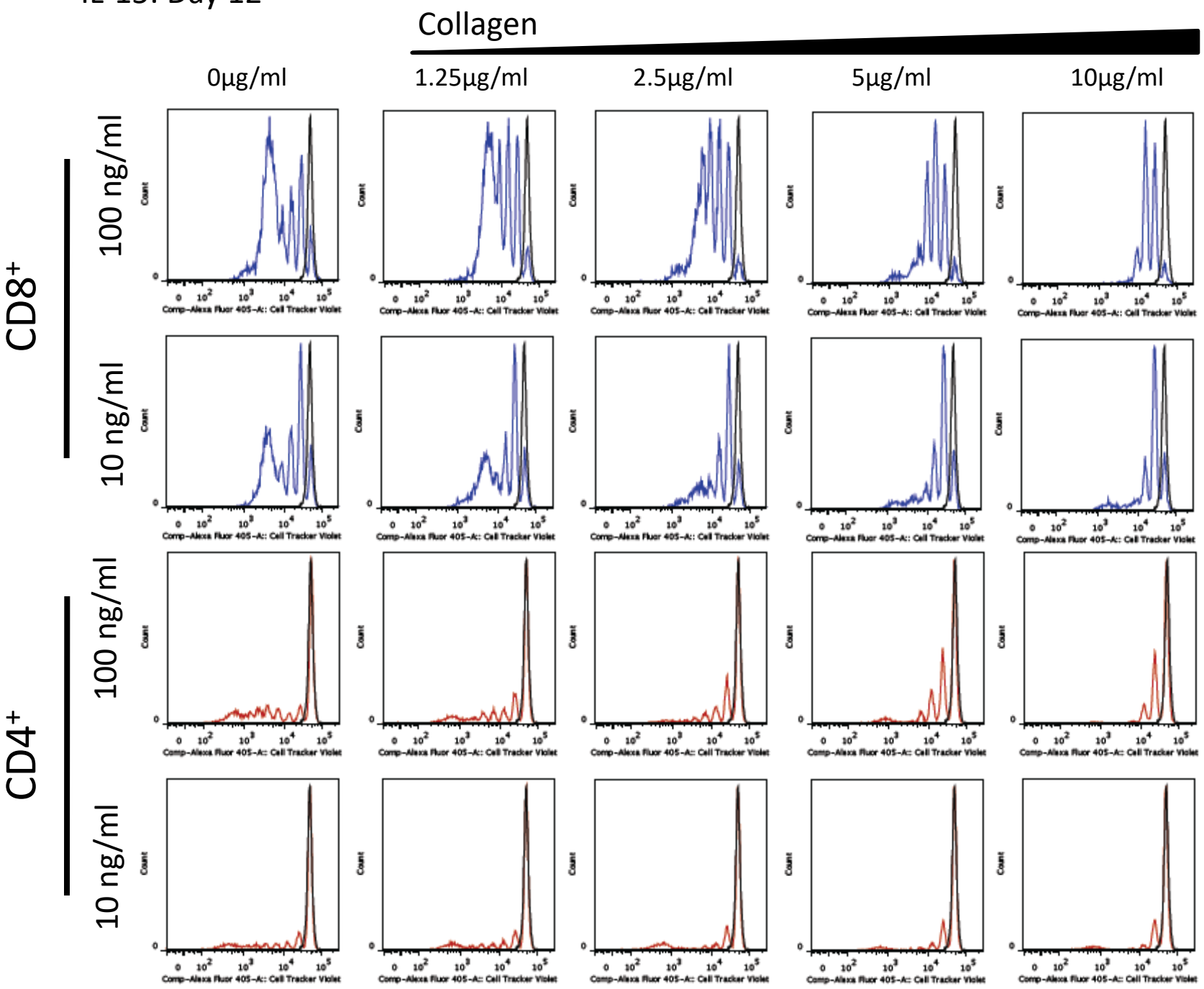

Generation 0: 1062
Generation 1: 450
Generation 2: 925
Generation 3: 1571
Generation 4: 1885
Generation 5: 1629
Generation 6: 861
Generation 7: 284

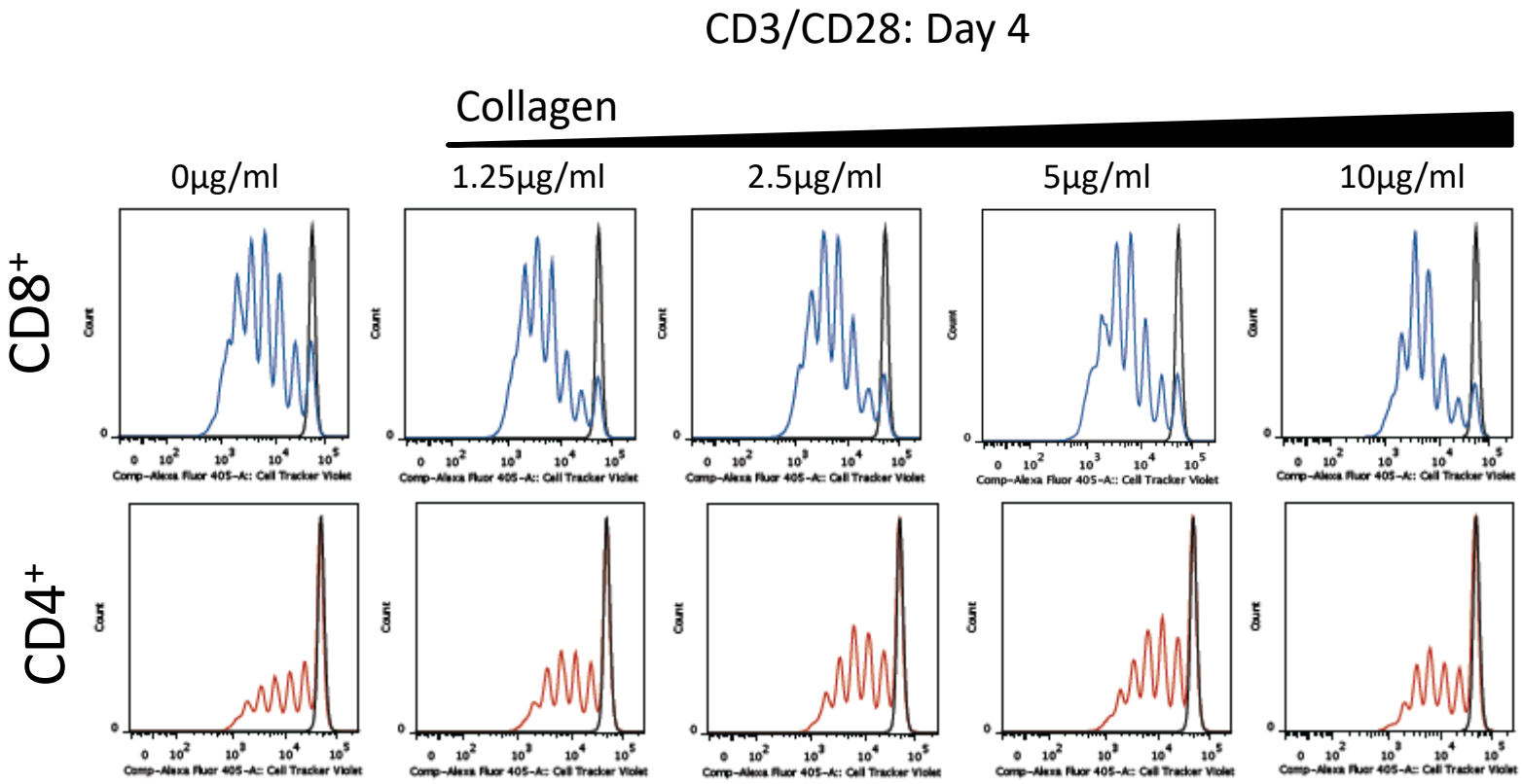
Percent Divided: 60.18
Proliferation Index: 106.85

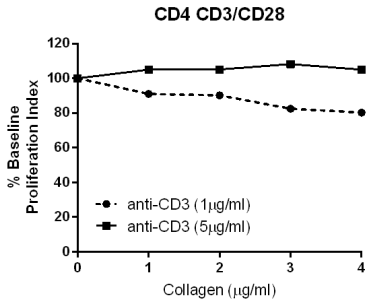

IL-2: Day 12
Collagen
0µg/ml
1.25µg/ml
2.5µg/ml
5µg/ml
10µg/ml
CD8+
CD4+
100 ng/ml
10 ng/ml
100 ng/ml
10 ng/ml
Count
Comp-Alexa Fluor 405-A: Cell Tracker Violet

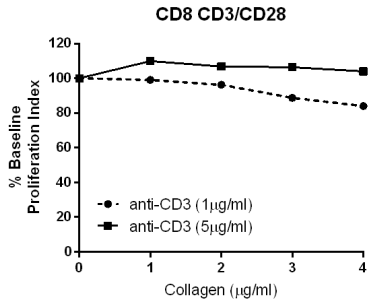

IL-7: Day 12
Collagen
0µg/ml
1.25µg/ml
2.5µg/ml
5µg/ml
10µg/ml
CD8+
CD4+
100 ng/ml
10 ng/ml
100 ng/ml
10 ng/ml
Count
Comp-Alexa Fluor 405-A: Cell Tracker Violet

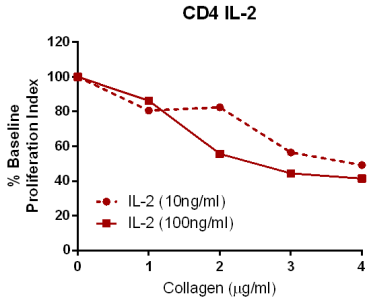
IL-15: Day 12

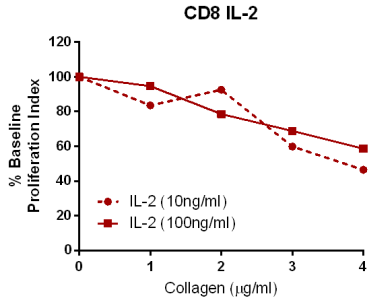

Collagen
0µg/ml
1.25µg/ml
2.5µg/ml
5µg/ml
10µg/ml
CD8+
CD4+
100 ng/ml
10 ng/ml
100 ng/ml
10 ng/ml
Count
Comp-Alexa Fluor 405-A: Cell Tracker Violet

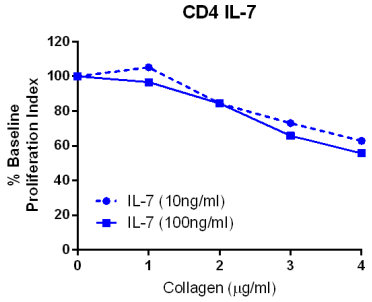

CD3/CD28: Day 4
Collagen
0µg/ml
1.25µg/ml
2.5µg/ml
5µg/ml
10µg/ml
CD8+
CD4+
Count
Comp-Alexa Fluor 405-A:: Cell Tracker Violet

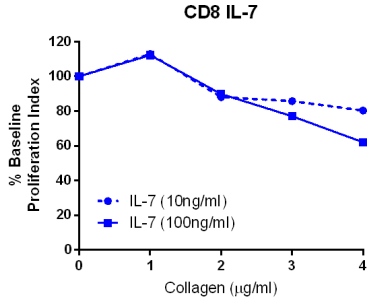

CD4 CD3/CD28
% Baseline Proliferation Index
120
100
80
60
40
20
0
anti-CD3 (1µg/ml)
anti-CD3 (5µg/ml)
0
1
2
3
4
Collagen (µg/ml)

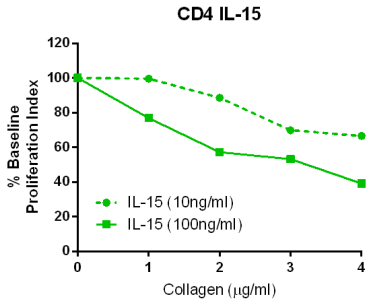

CD8 CD3/CD28
% Baseline Proliferation Index
120
100
80
60
40
20
0
anti-CD3 (1µg/ml)
anti-CD3 (5µg/ml)
0
1
2
3
4
Collagen (µg/ml)

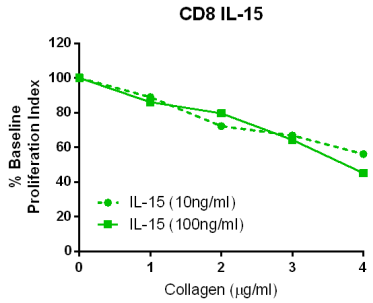

CD4 IL-2
% Baseline Proliferation Index
120
100
80
60
40
20
0
IL-2 (10ng/ml)
IL-2 (100ng/ml)
0
1
2
3
4
Collagen (µg/ml)


CD8 IL-2
% Baseline Proliferation Index
120
100
80
60
40
20
0
IL-2 (10ng/ml)
IL-2 (100ng/ml)
0
1
2
3
4
Collagen (µg/ml)

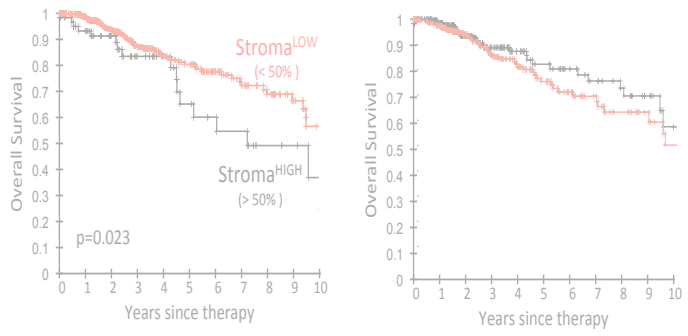

CD4 IL-7
% Baseline Proliferation Index
120
100
80
60
40
20
0
IL-7 (10ng/ml)
IL-7 (100ng/ml)
0
1
2
3
4
Collagen (µg/ml)


CD8 IL-7
% Baseline Proliferation Index
120
100
80
60
40
20
0
IL-7 (10ng/ml)
IL-7 (100ng/ml)
0
1
2
3
4
Collagen (µg/ml)

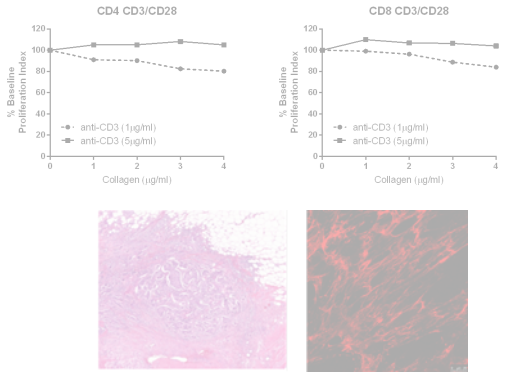

CD4 IL-15
% Baseline Proliferation Index
120
100
80
60
40
20
0
IL-15 (10ng/ml)
IL-15 (100ng/ml)
0
1
2
3
4
Collagen (µg/ml)


CD8 IL-15
% Baseline Proliferation Index
120
100
80
60
40
20
0
IL-15 (10ng/ml)
IL-15 (100ng/ml)
0
1
2
3
4
Collagen (µg/ml)

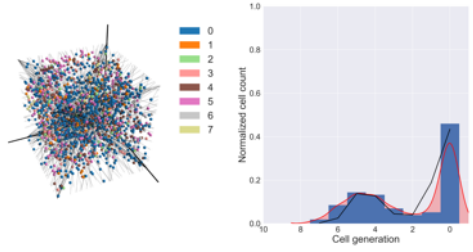
## Statistical Analysis
## TCGA / TCC



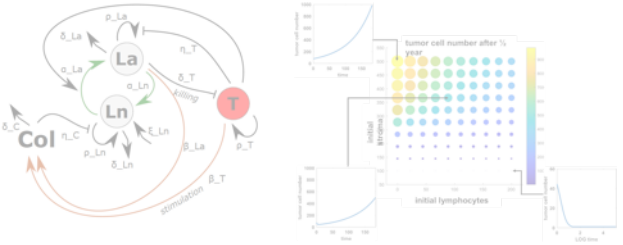

## Experimental data




## Agent Based Models

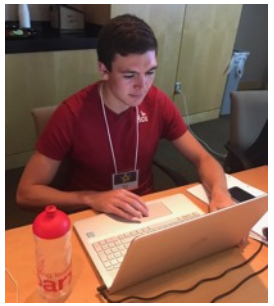


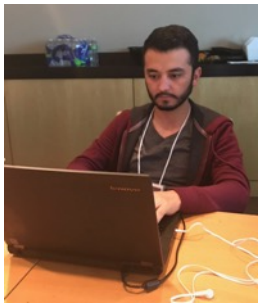

## ODE Model



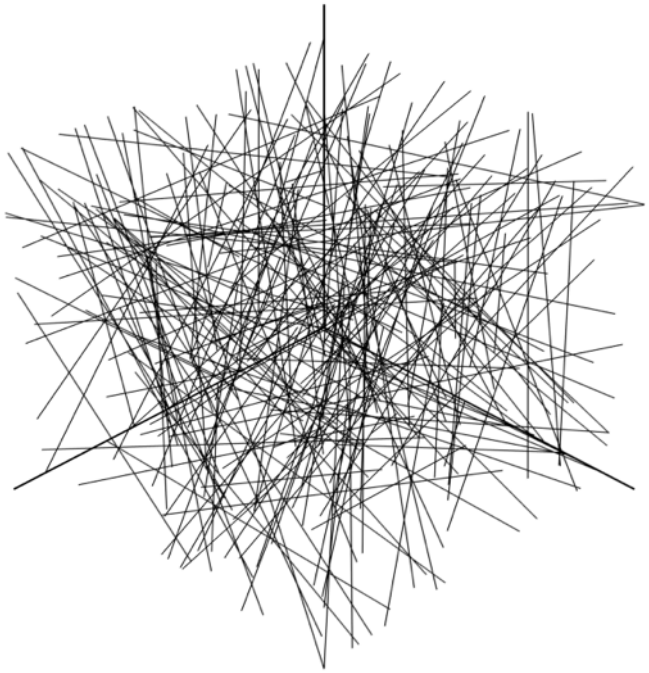

## Proposal

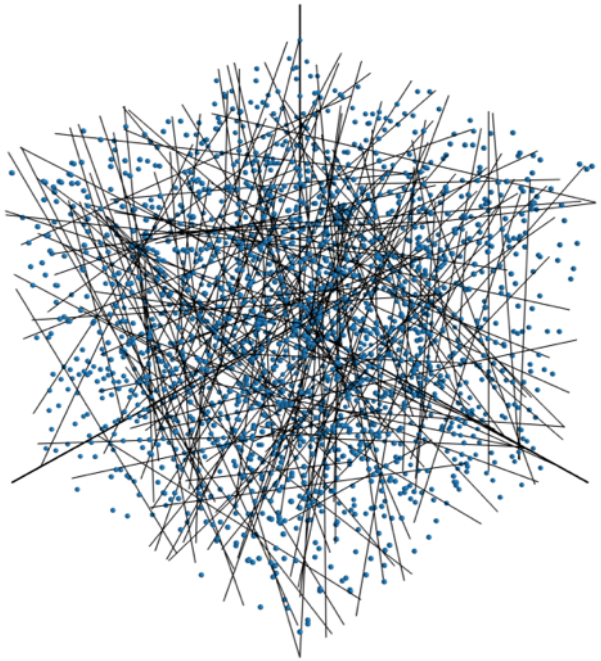
# ABM rules



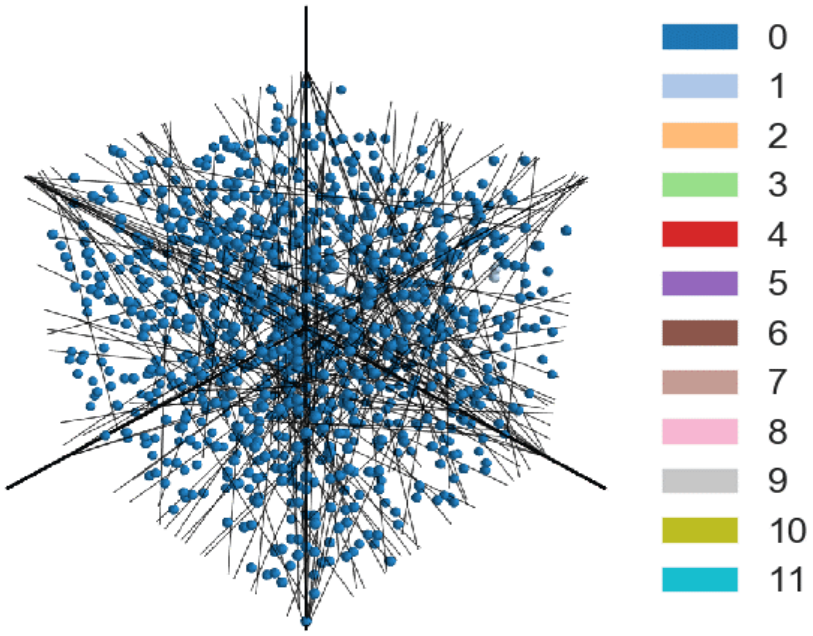


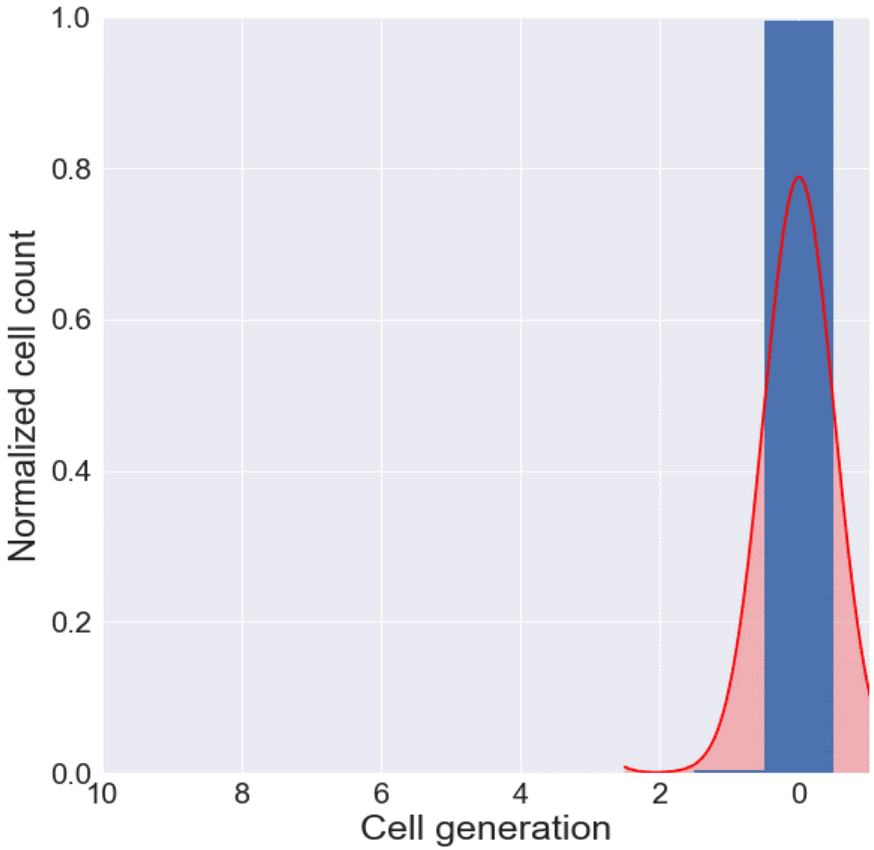
- Collagen contact inhibits the cell division
- Cells move by Brownian motion
- Once a cell divides both parent and daughter cell become a generation older
- We track the generation distribution at the end of the simulation





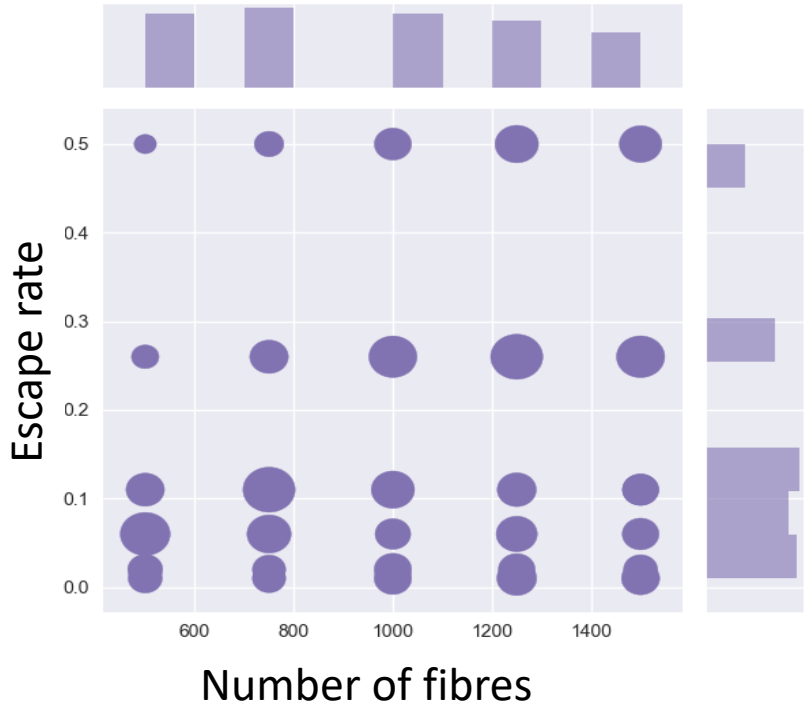
# An example simulation

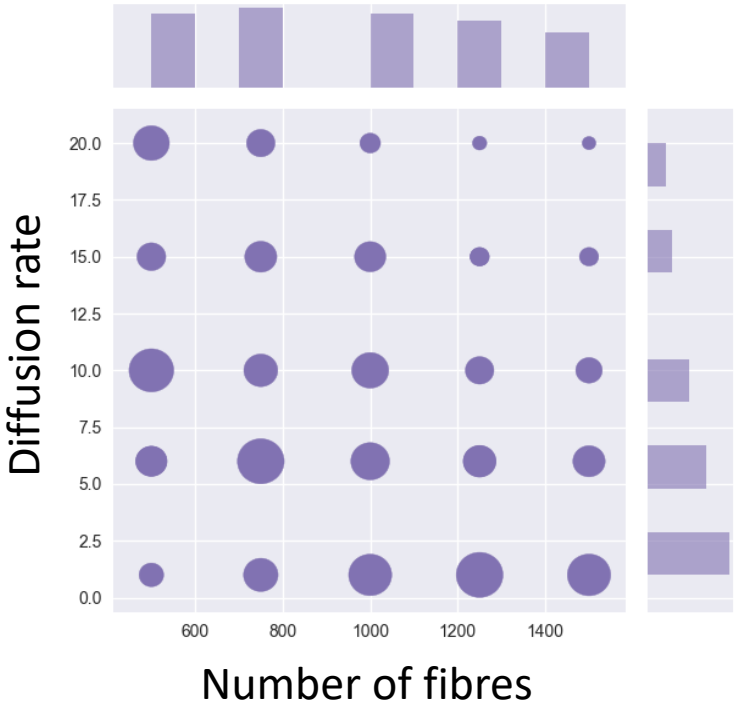


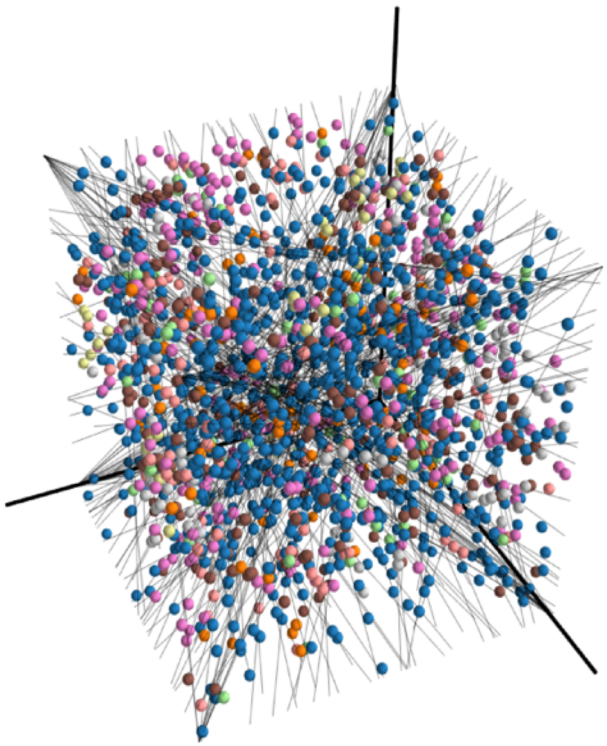
# Tweakable parameters

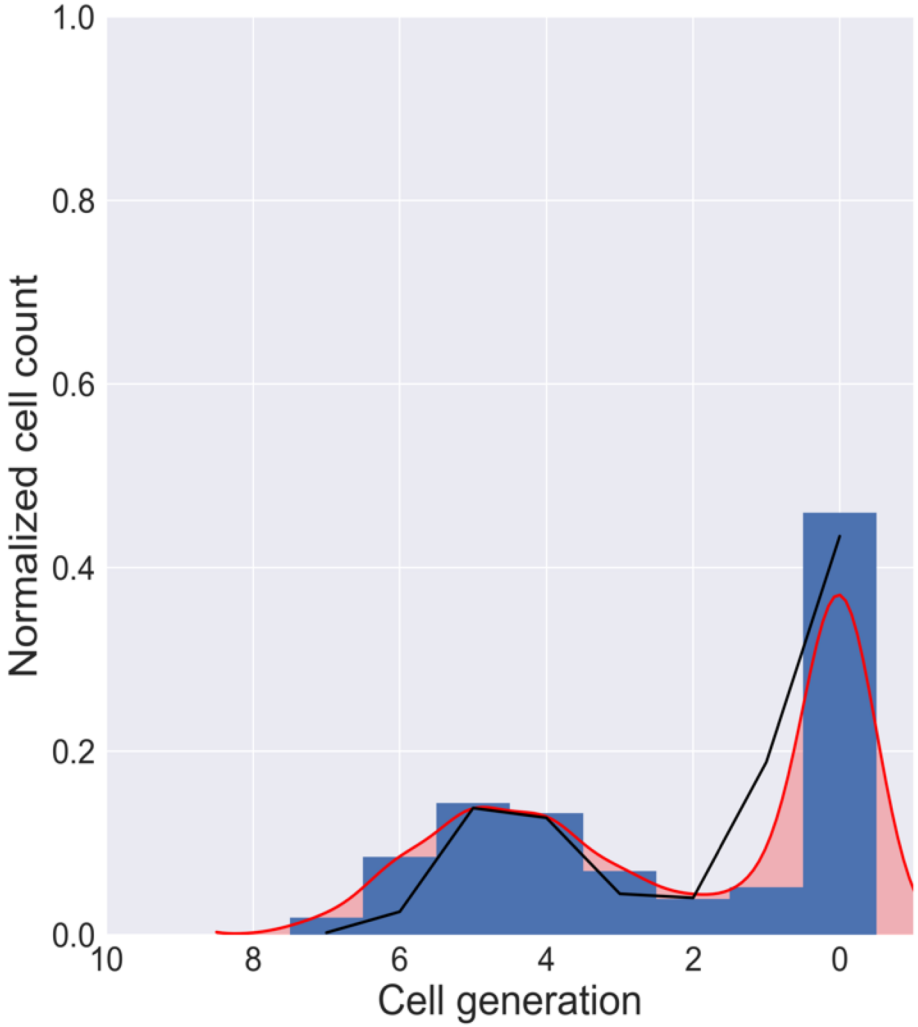
- How often cells move per generation?
- How fast the cells escape after collagen contact.
- Collagen density in the well.



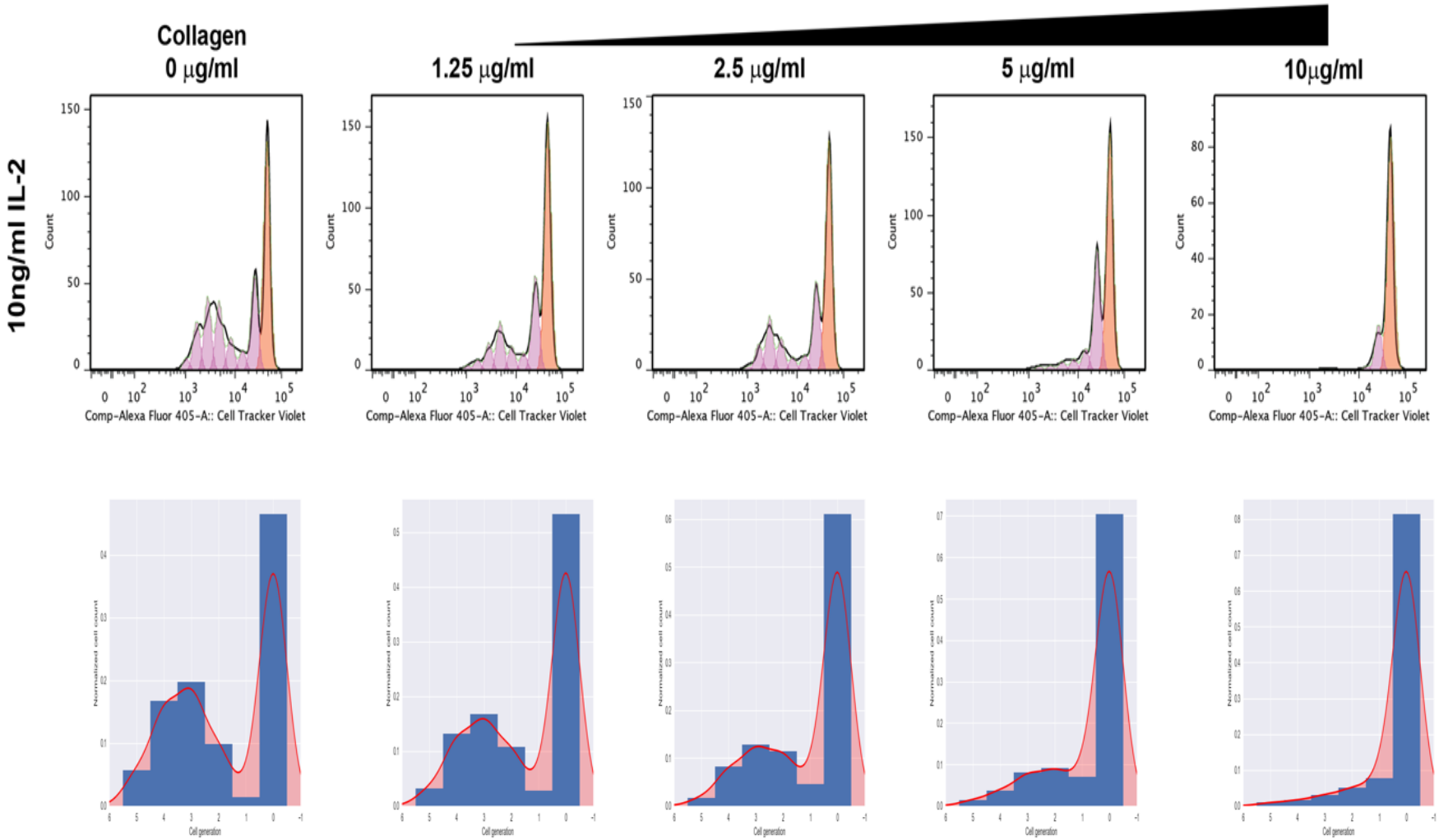



# Best fit for 2.5 $\mu g/mL$ collagen



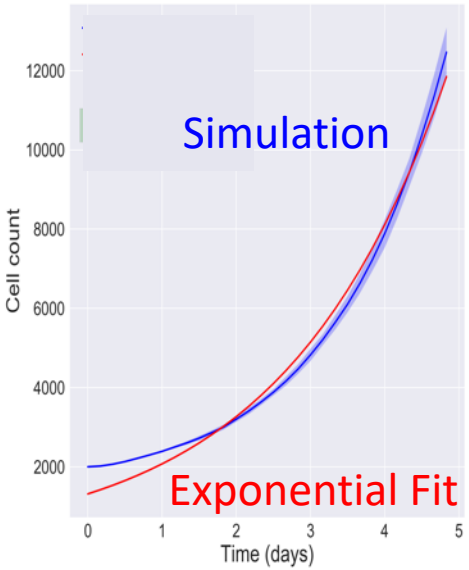
# Best fit for entire IL-2 assay



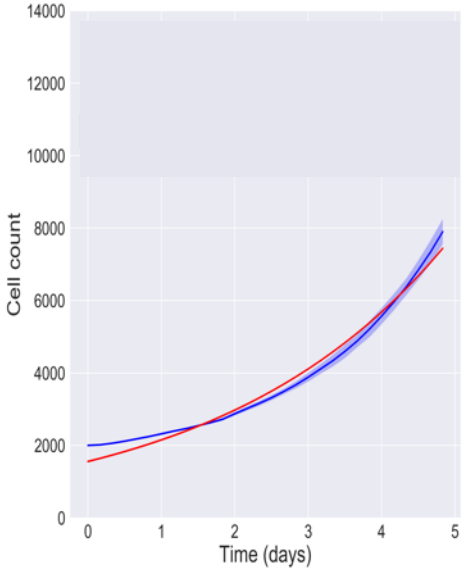
# Determine functional response of T cell proliferation (R) to collagen density

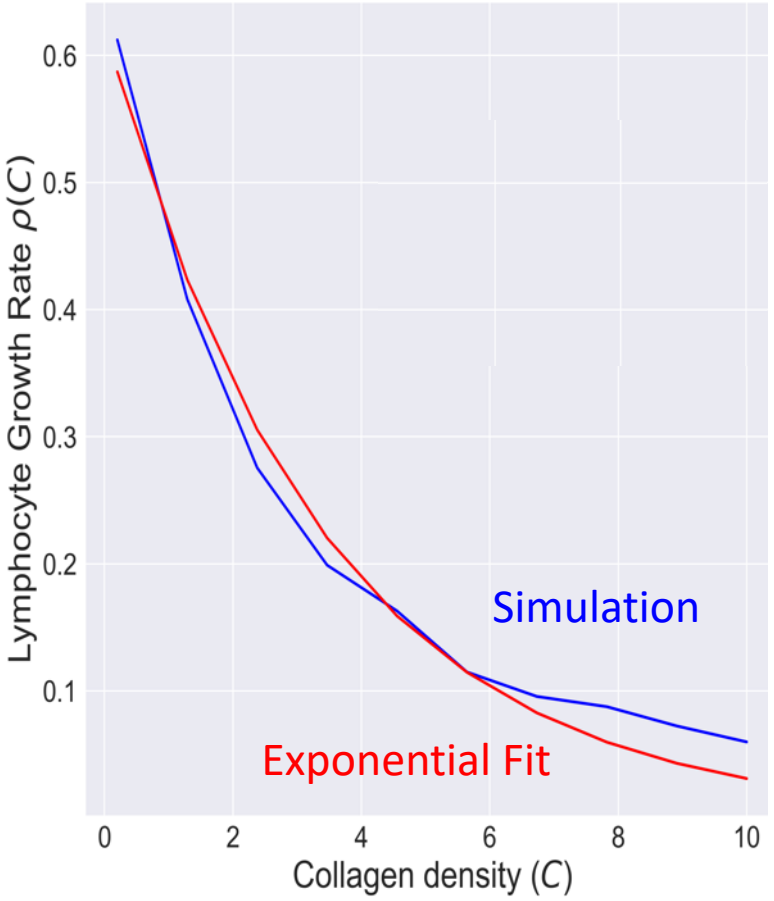
$$\frac{dR}{dt} = p(C)R$$

$C_{LOW}$

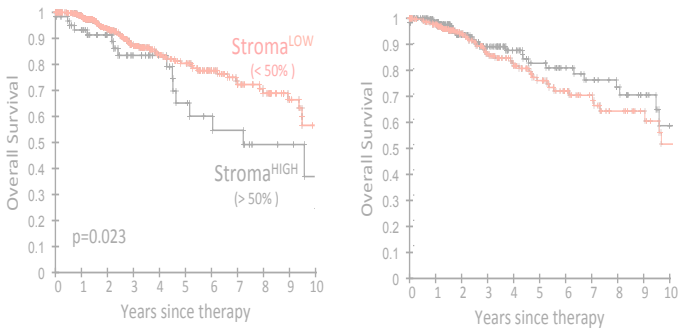



$C_{HIGH}$

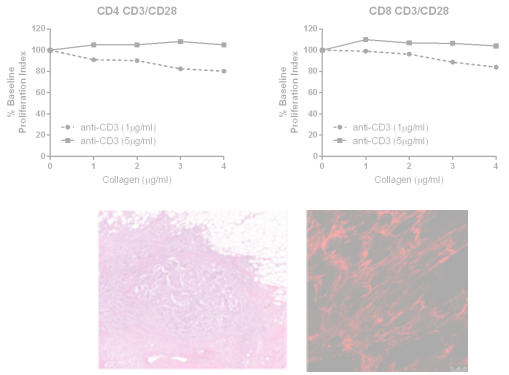


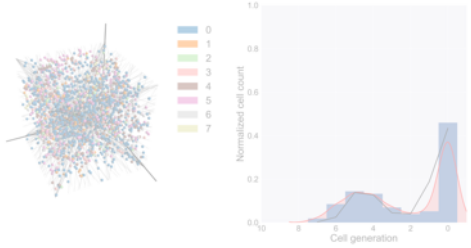
## Statistical Analysis
## TCGA / TCC



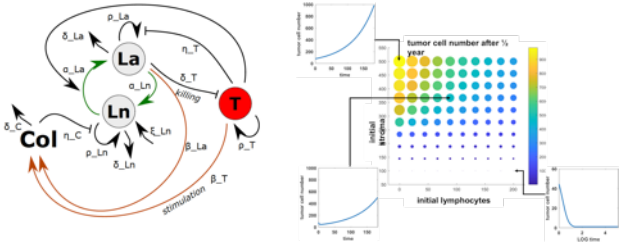

## Experimental data




## Agent Based Models

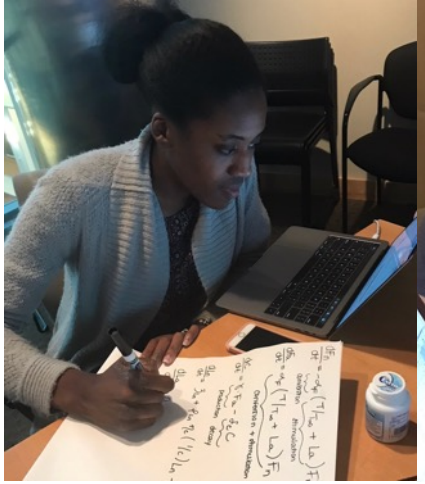


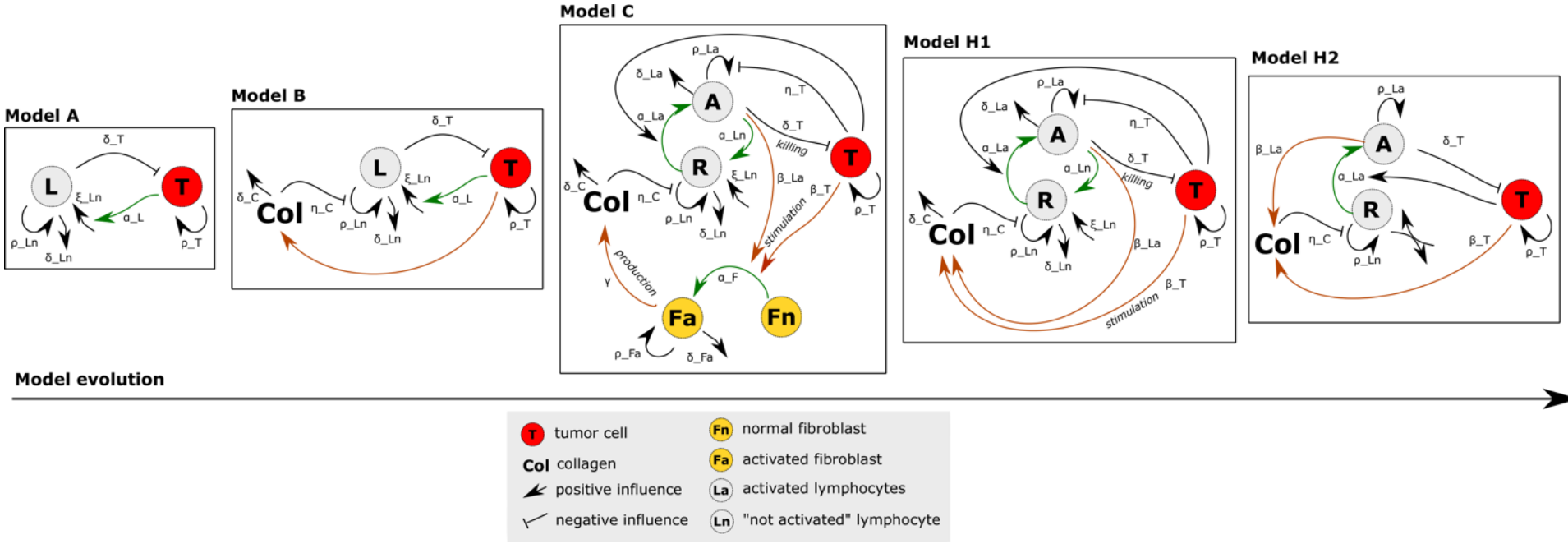

## ODE Model




## Proposal

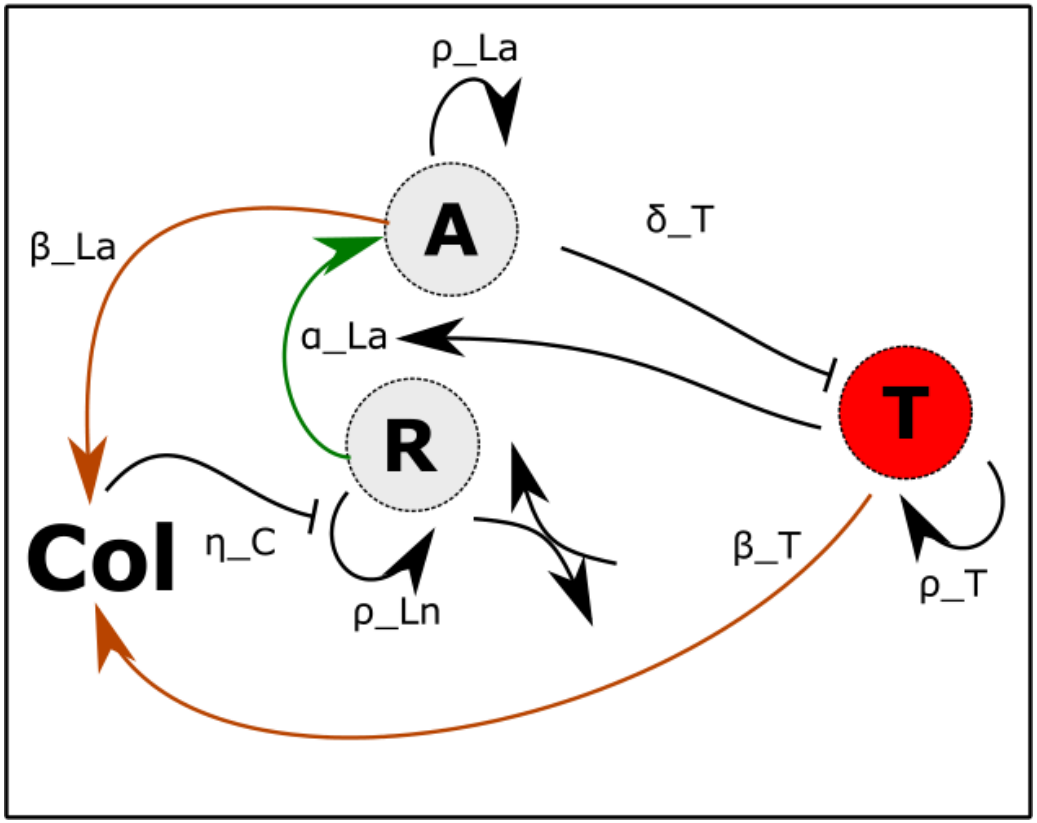
# Model evolution





# Final model

**Model H2**

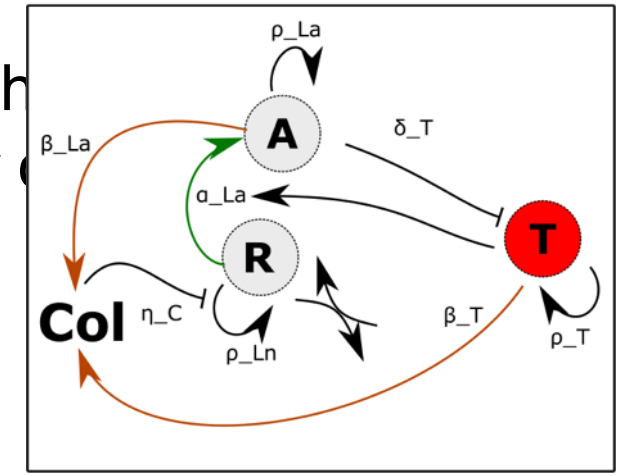



Δ collagen $\frac{dC}{dt} = \beta_T T + \beta_A A$ = stimulation by tumor + stimulation by activated lymphocytes

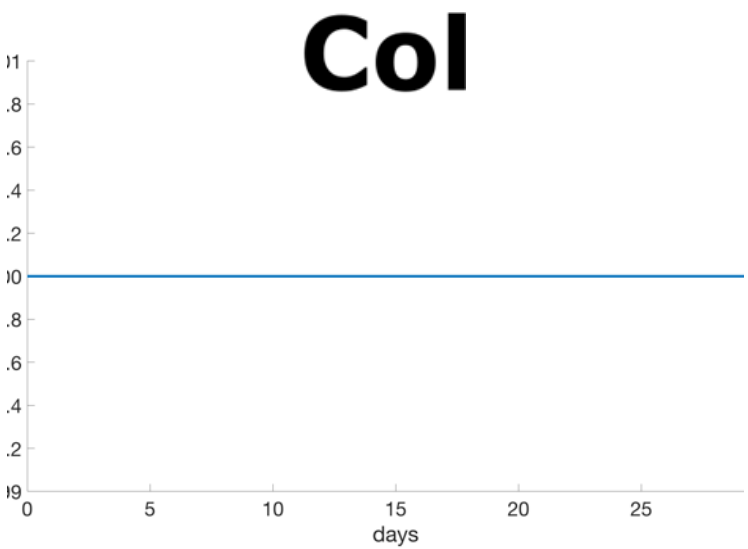
Δ resting lymphocytes $\frac{dR}{dt} = \psi(R^* - R) + \rho_R e^{-\eta C} - \alpha T R$ = net influx & regress + proliferation decreased by collagen − convert resting to active

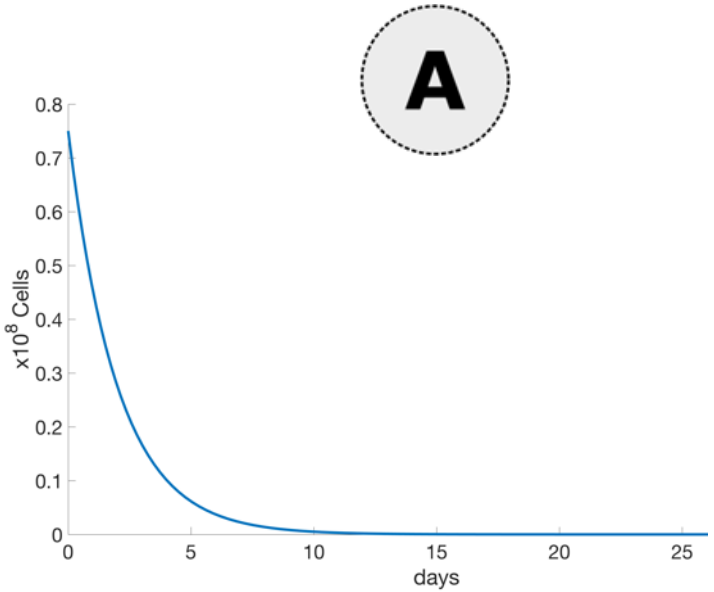
Δ active lymphocytes $\frac{dA}{dt} = \alpha T R + \rho_A A \cdot f(T) - \delta_A A$ = convert resting to active + proliferation in presence of tumor − death of active lymphocytes

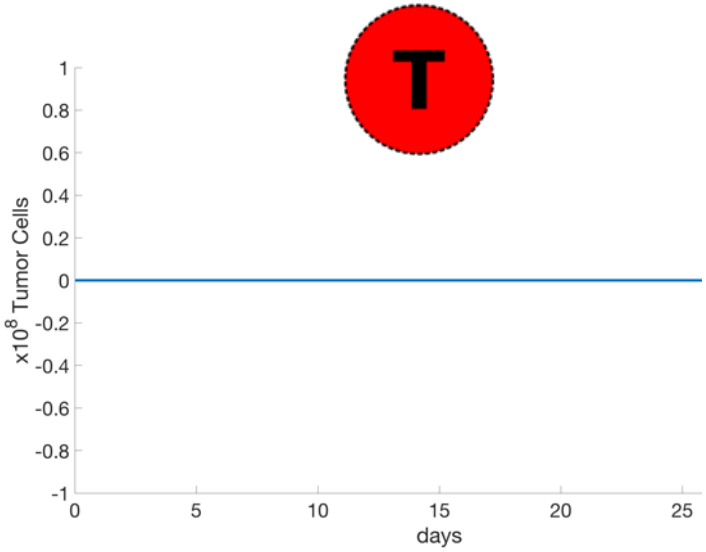
Δ tumor cells $\frac{dT}{dt} = \rho_T T - \delta_T A T$ = proliferation of tumor − death tumor

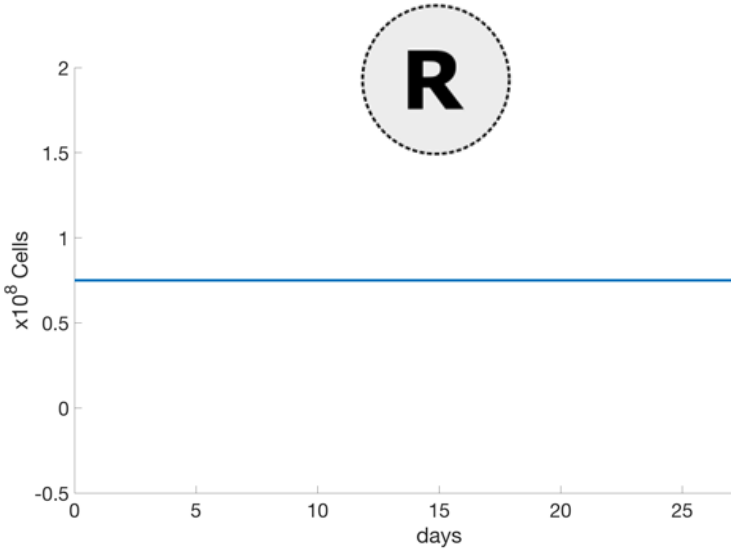


# Homeostasis – no tumor

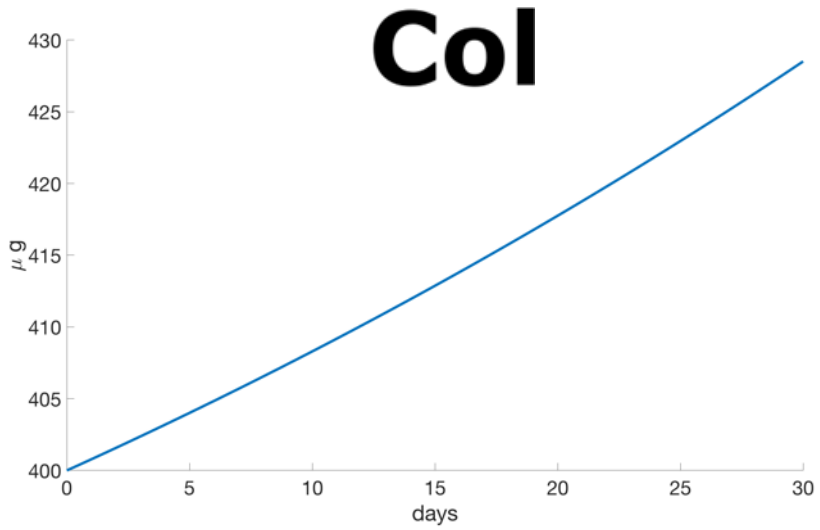


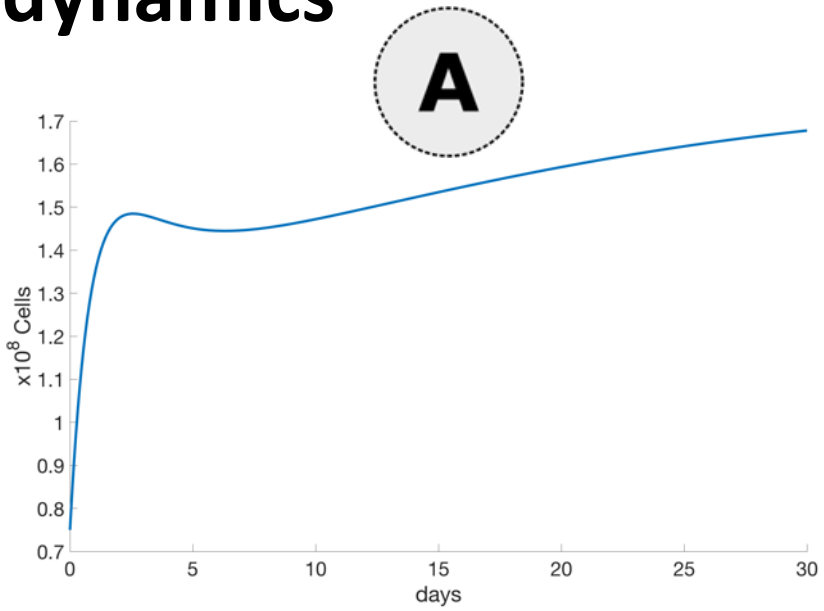



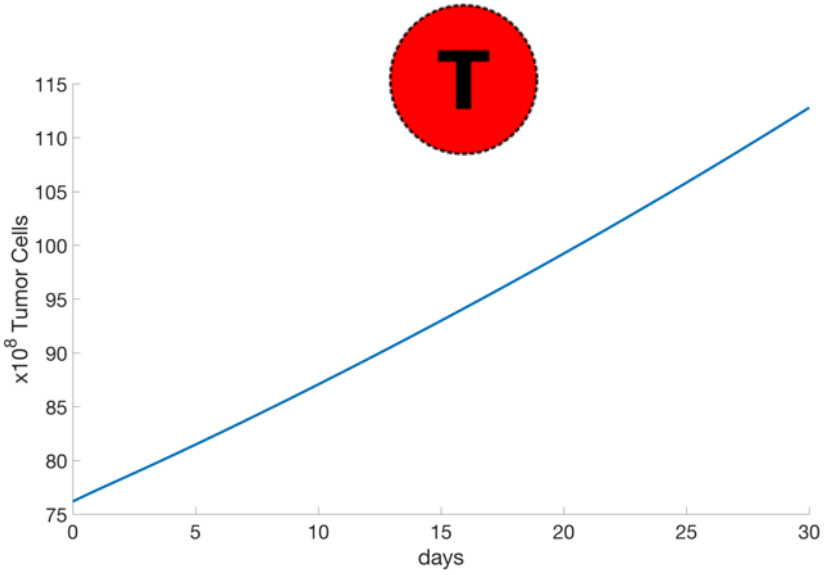



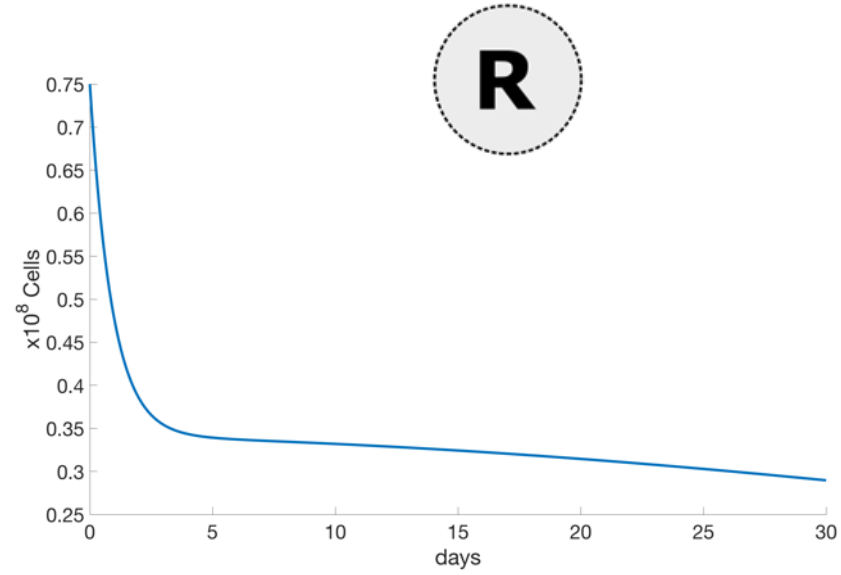



# Tumor growth dynamics

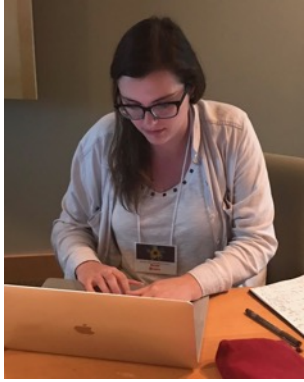


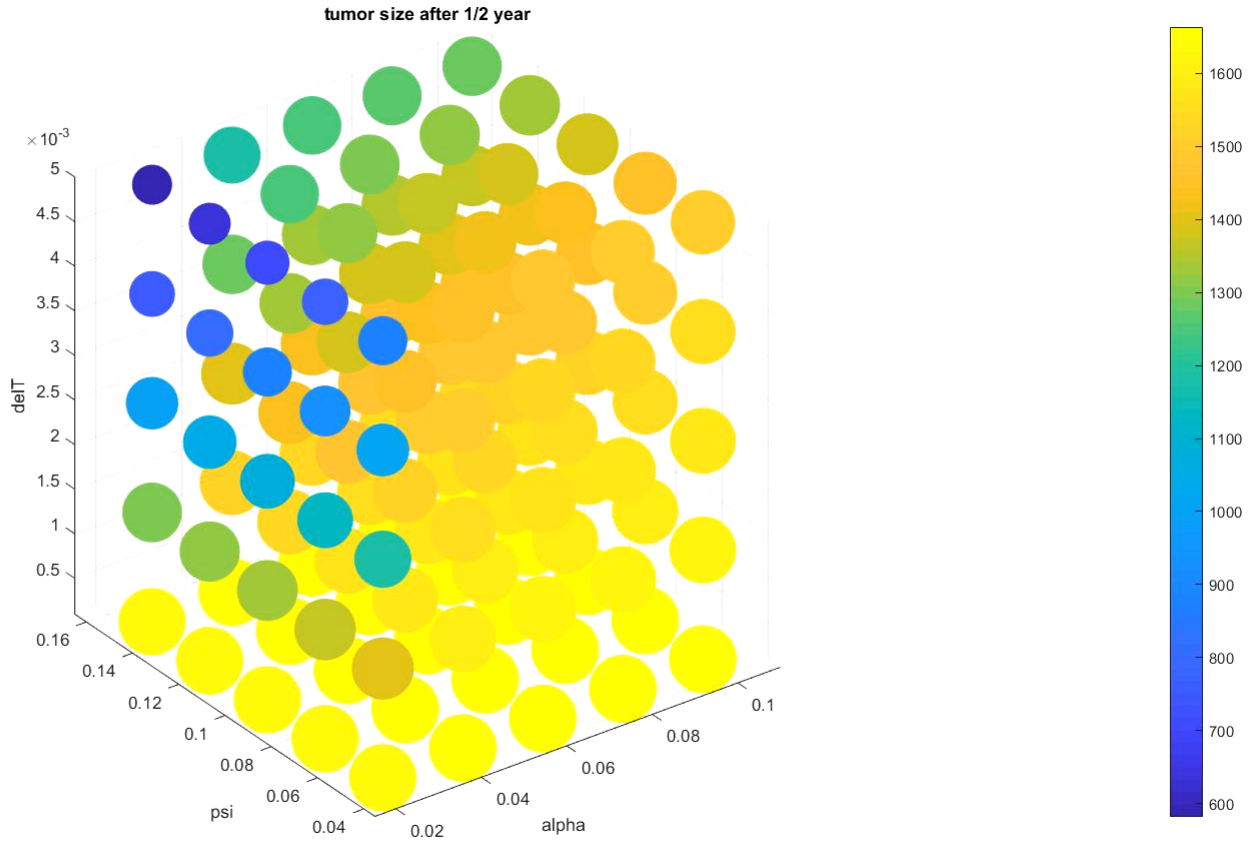



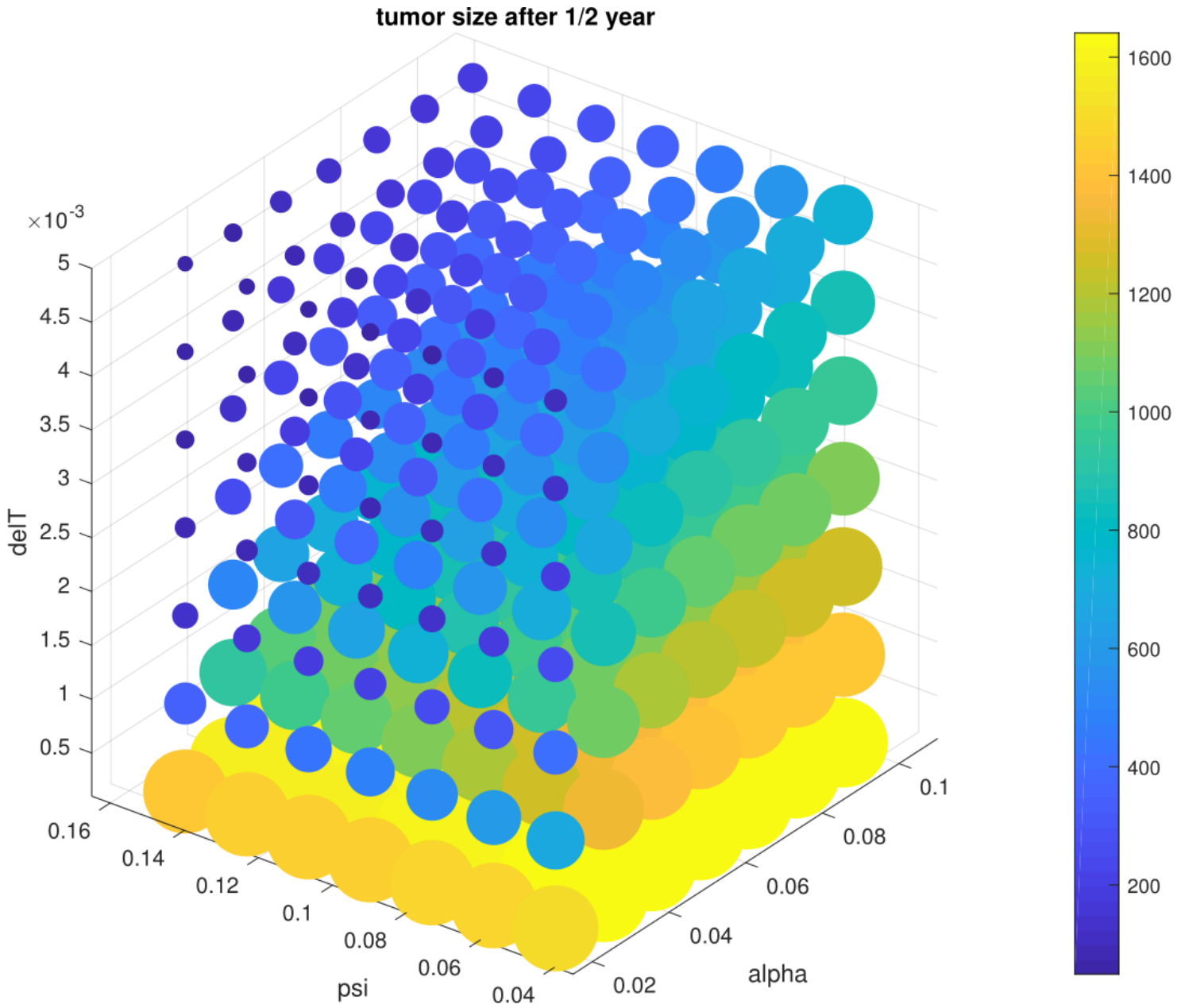



# Sensitivity to parameters 1/2

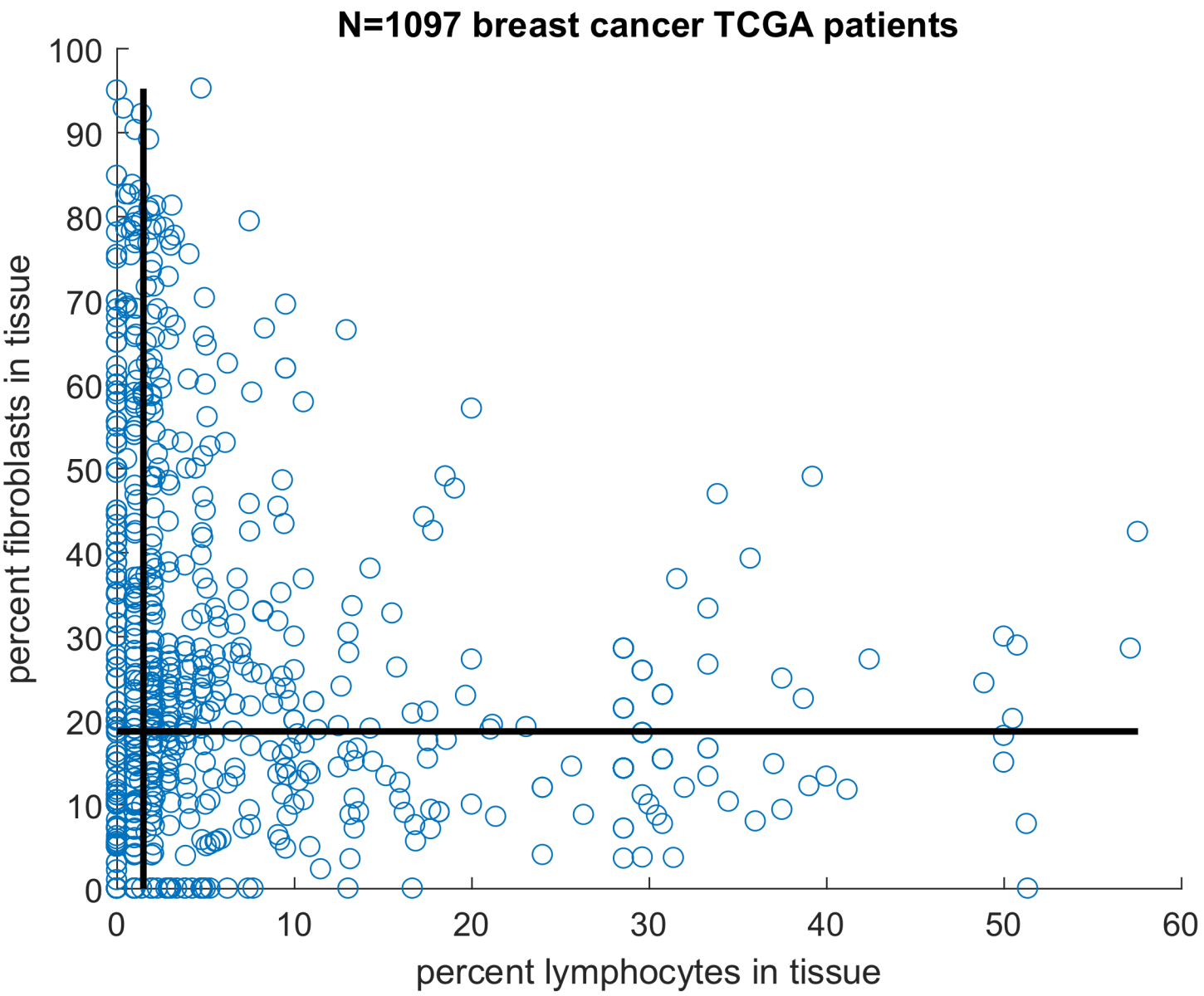




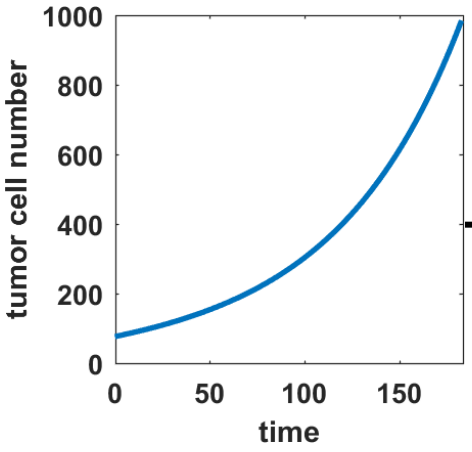
# Sensitivity to parameters 2/2

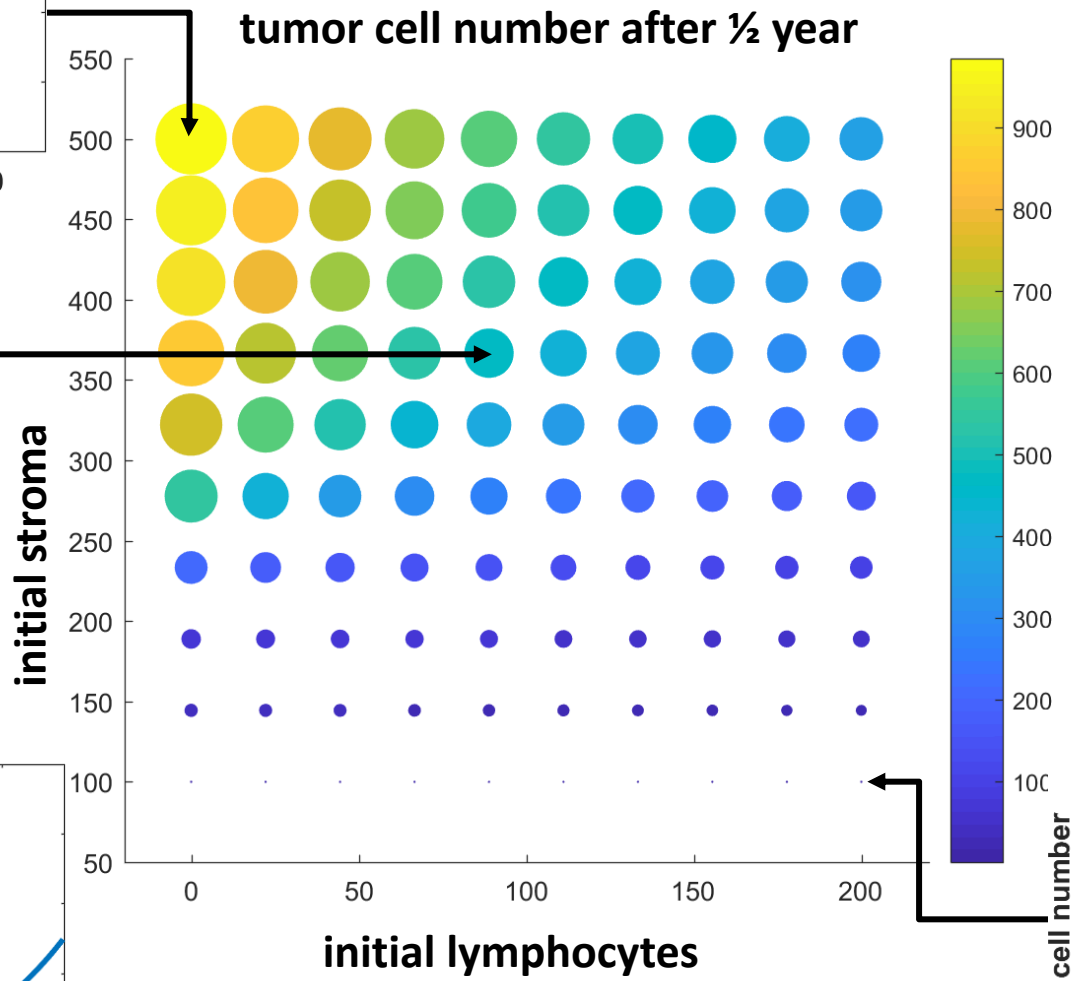


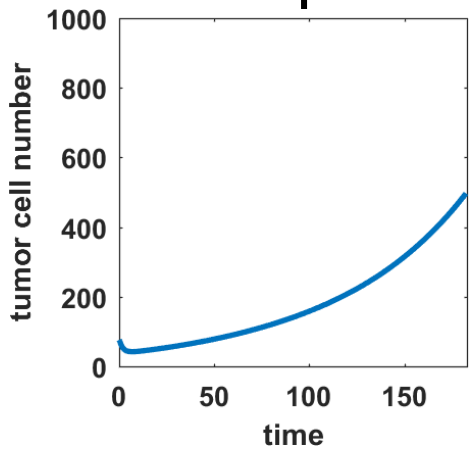
# Stroma and lymphocytes in patients

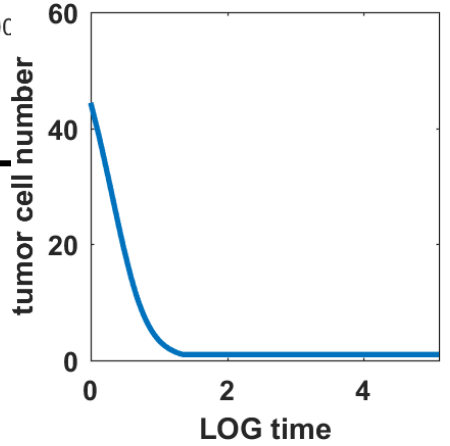


# Sensitivity to initial conditions

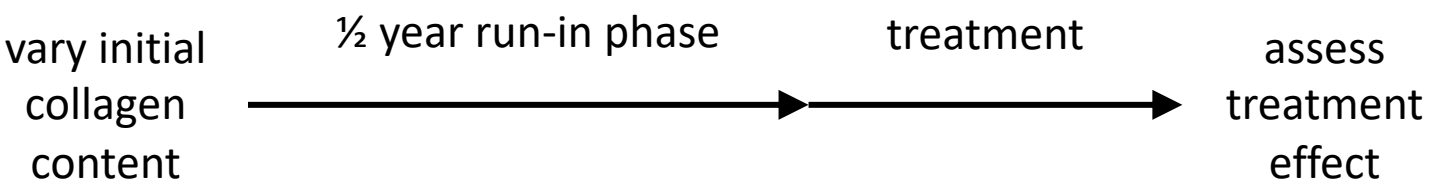


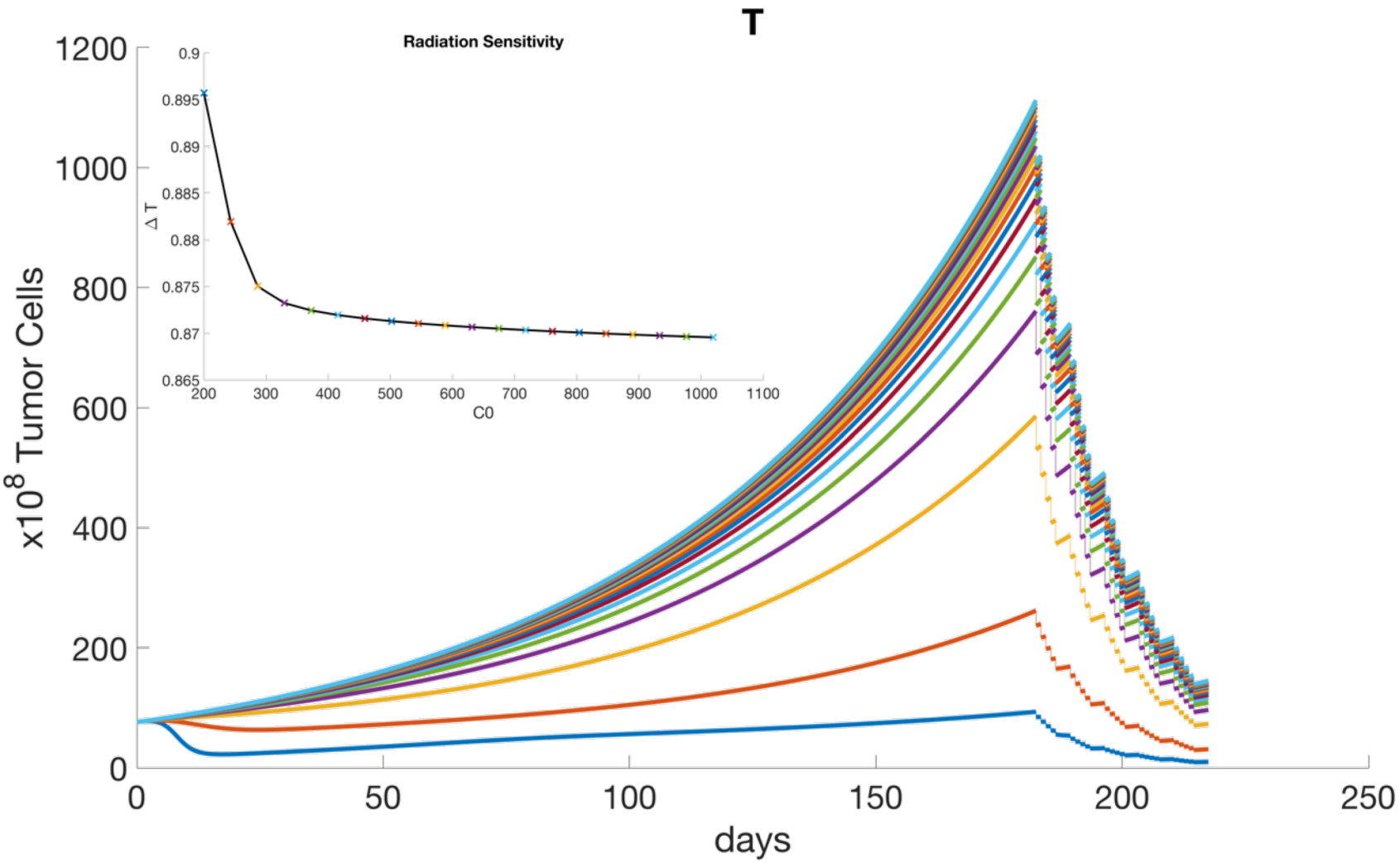



# Treatment simulation



# Radiotherapy simulation

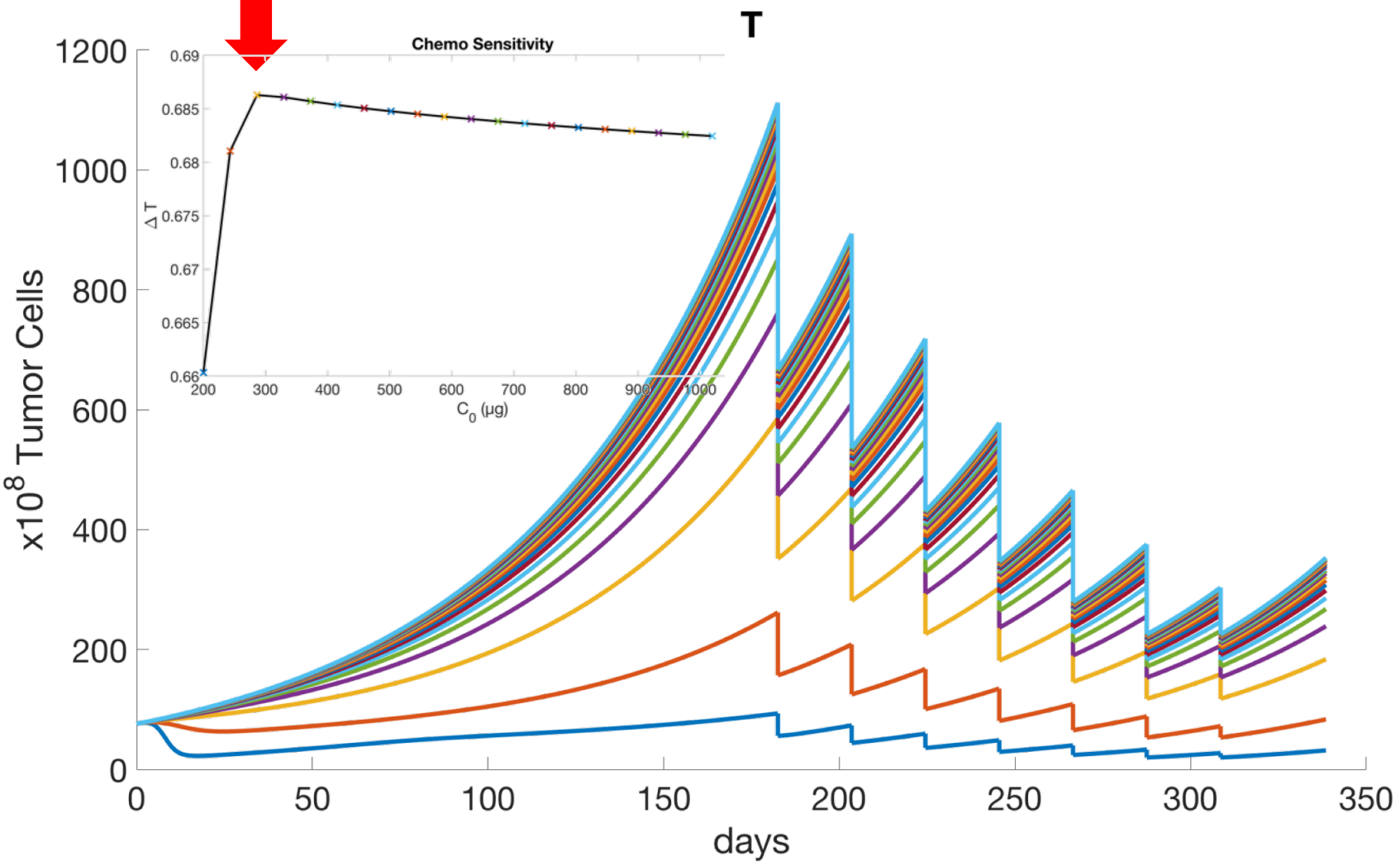
**5x5 doses of 2 Gy**



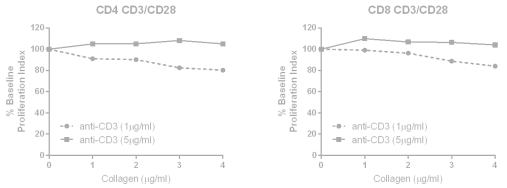
# Chemotherapy simulation

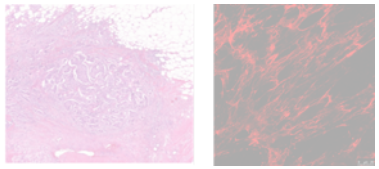
**Docetaxel, given every 3 wk**

**highest Chemo sensitivity**

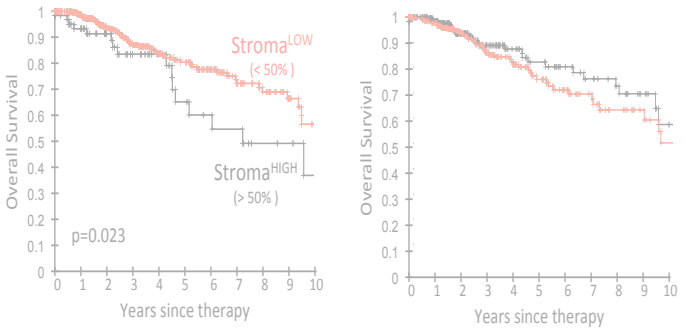


## Experimental data

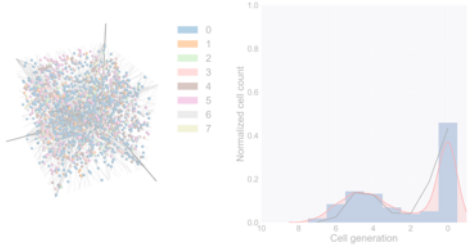


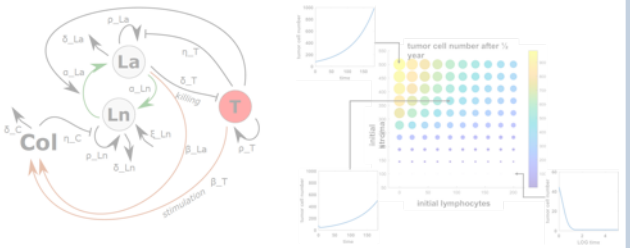
## Statistical Analysis
## TCGA / TCC




## Agent Based Models




## ODE Model

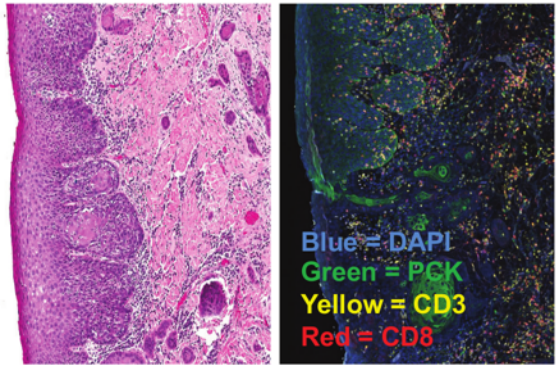



## Proposal

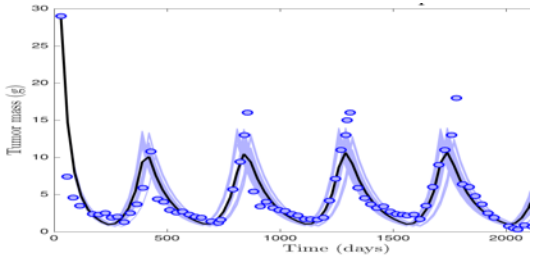
**Central Hypothesis**: Treatment response and ultimately outcome may be linked to the *patient-specific Tumor-Stroma-Immune Ecosystem (TSIE)* prior to and its evolution during therapy

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

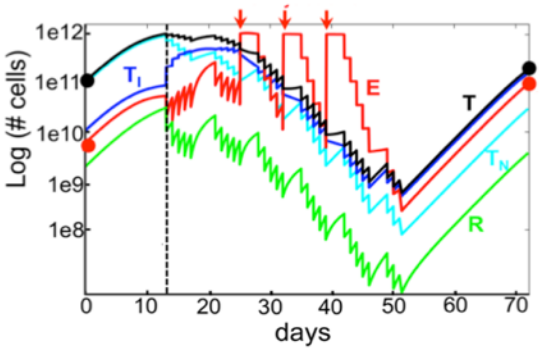
**1A)** Assess *TSIE* composition (multiplex immunohistochemistry, gene expression) in retrospecitve TCC biopsy samples and correlate *TSIE* composition with treatment outcome (pCR).




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

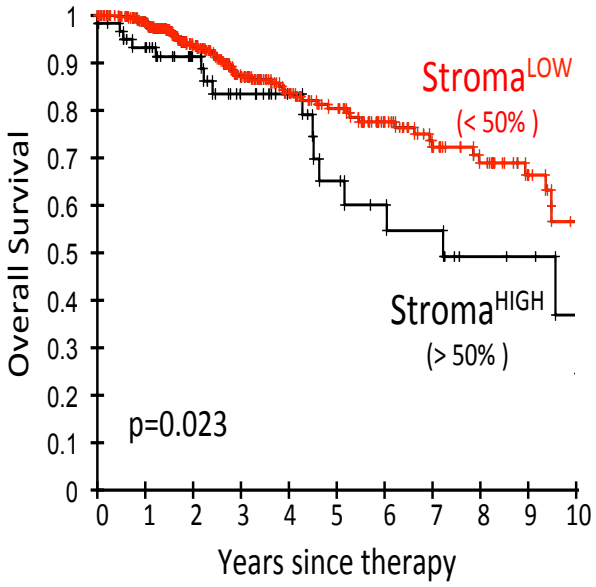



**AIM 2. Predict optimal treatment for immune activation and tumor control.** Simulate if NCT can be escalated where needed and de-escalated where possible.



# Patient impact

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



# 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

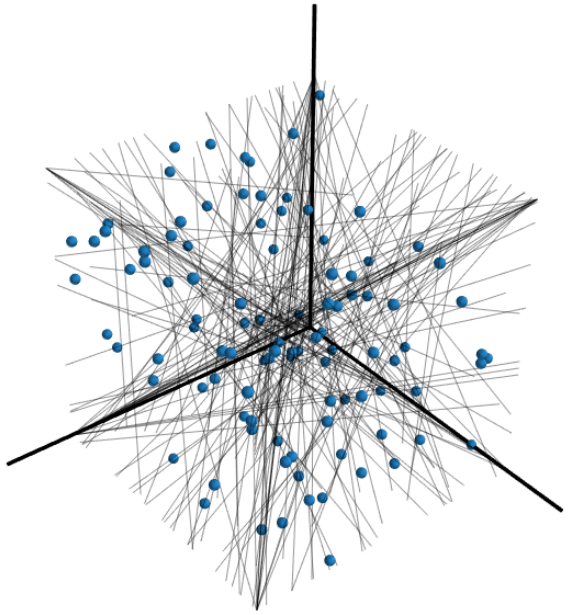
- AACR presentation, $2.5K
  - – (registration, airfare, lodging)
- Cancer Research publication, $2.5K

$50K

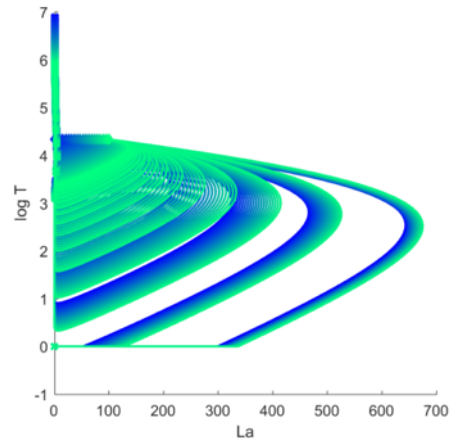
# Thank You.

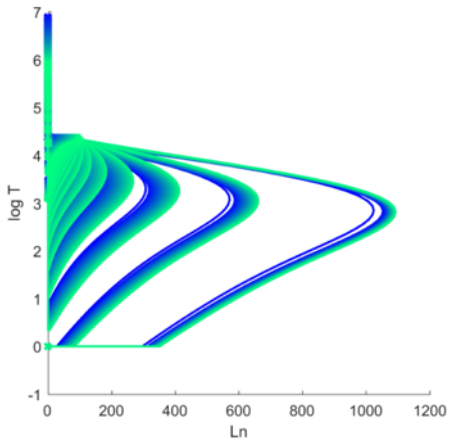
IMO
PSOC
Moffitt Cancer Center
Sandy Anderson
Danae Paris

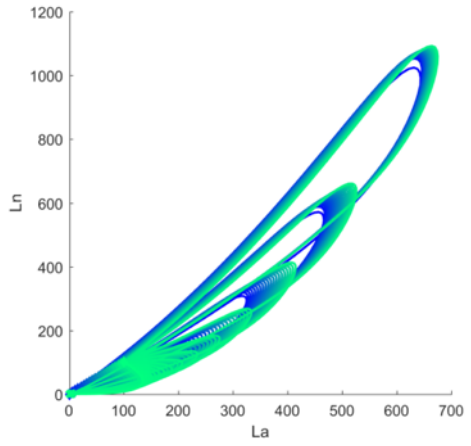
Jai Alai

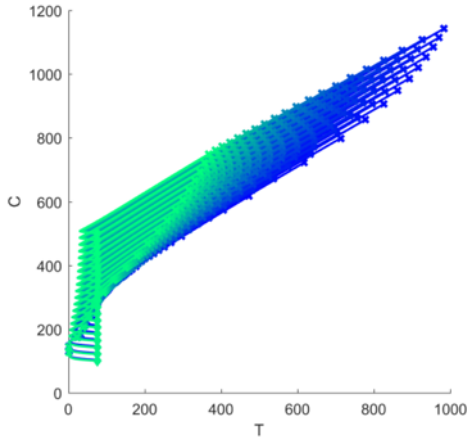


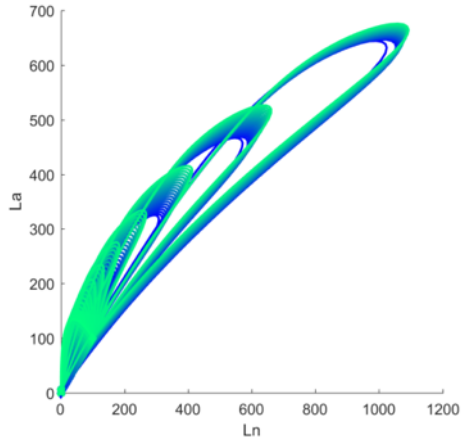
# Evolution of dynamic immune state 1/2

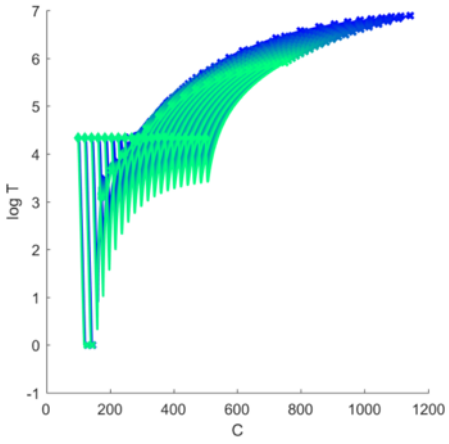


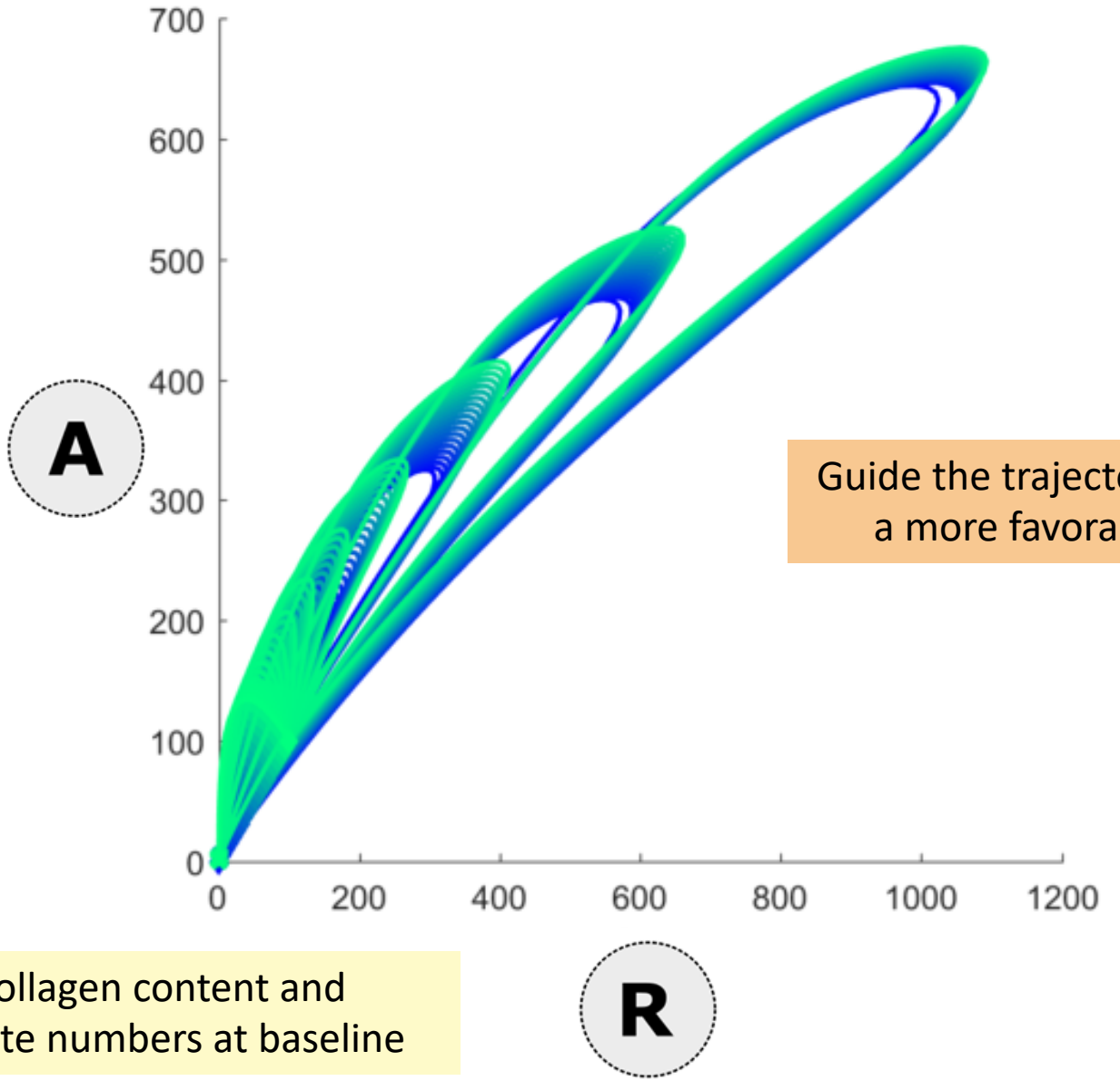



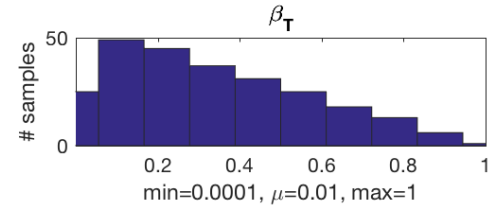
# Evolution of dynamic immune state 2/2

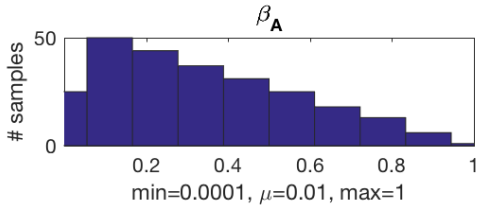


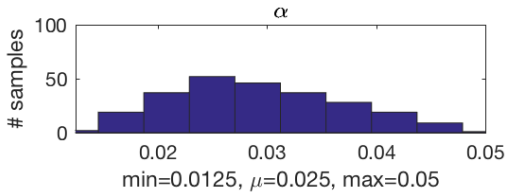

Guide the trajectory towards a more favorable state

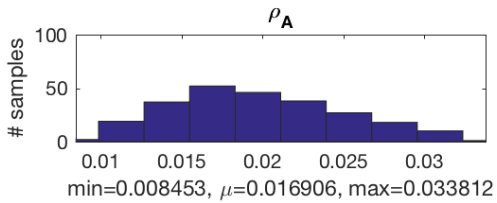
Vary collagen content and lymphocyte numbers at baseline

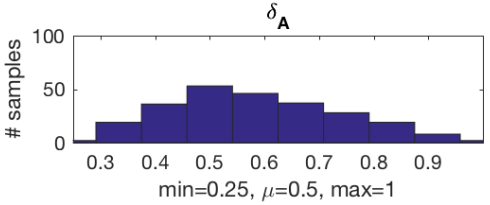
This does NOT include eta and Rho_R

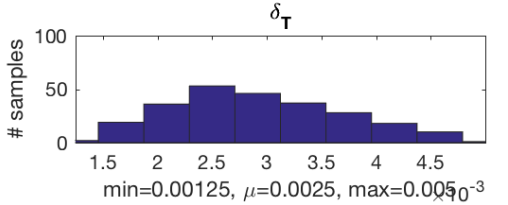


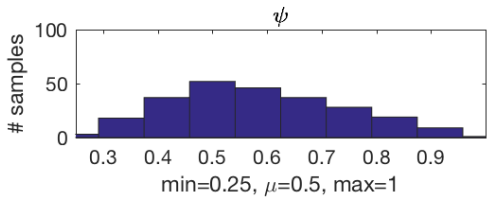



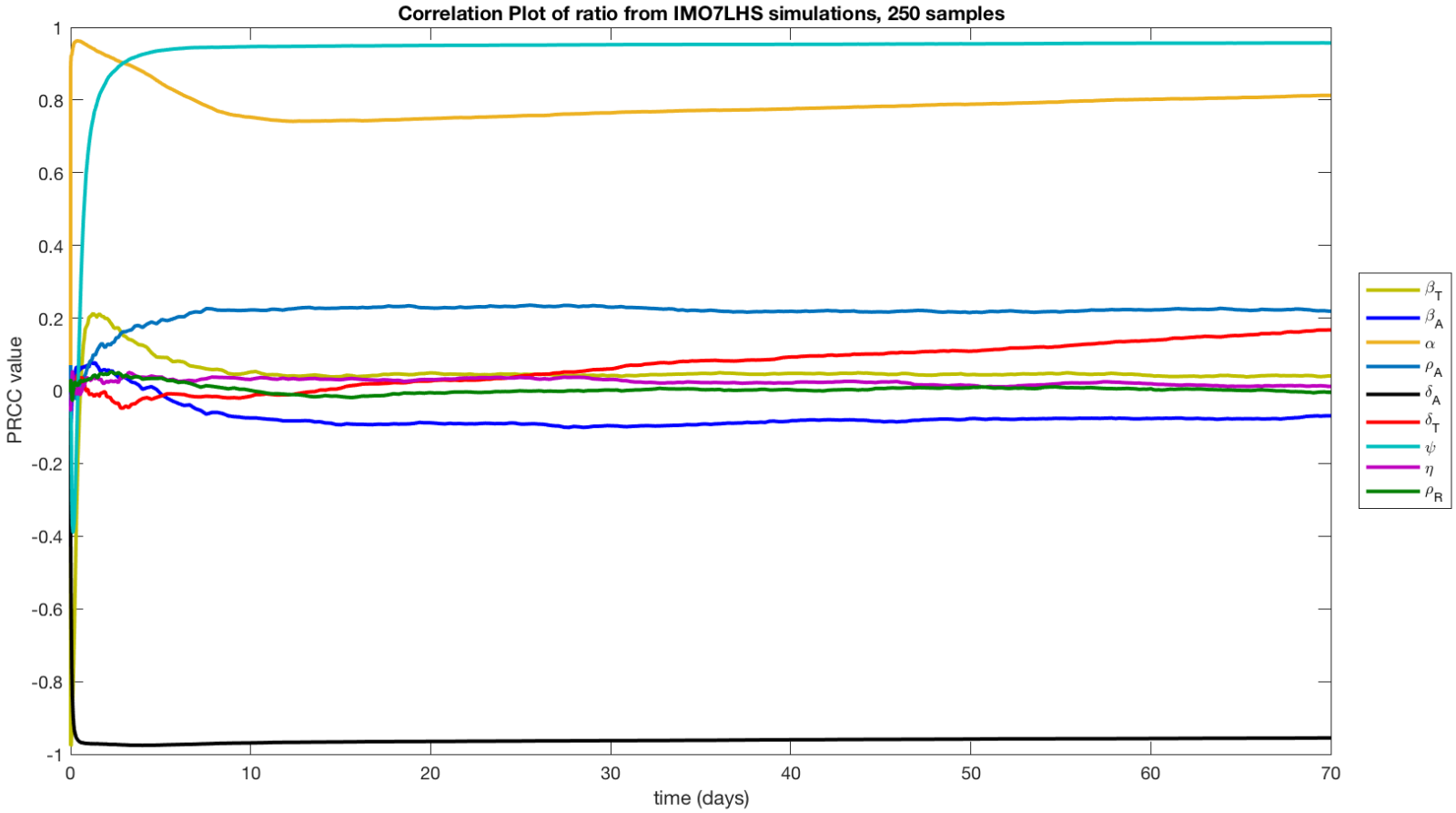



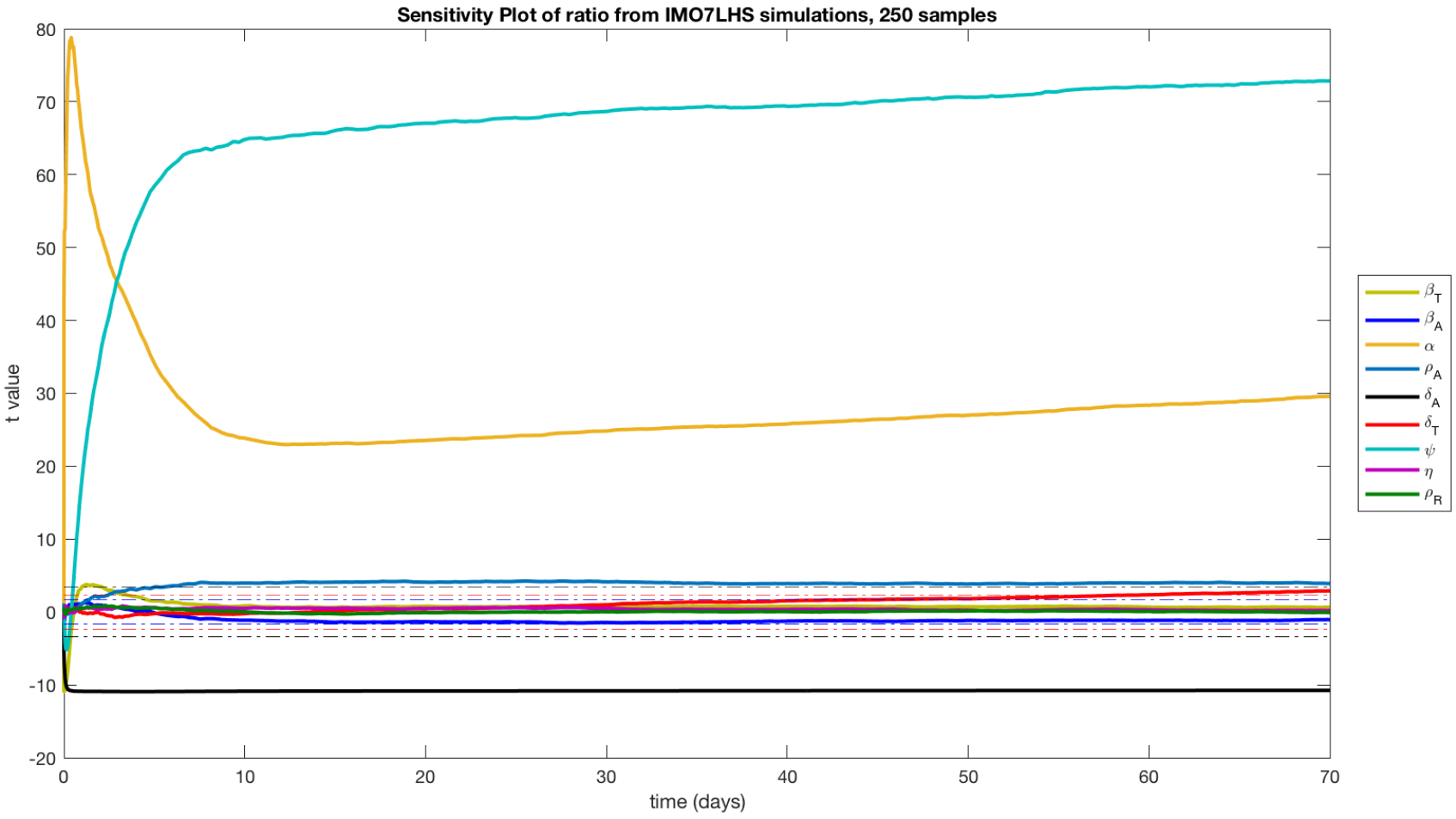



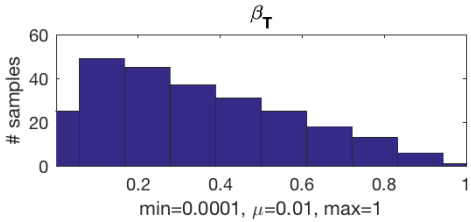
This DOES include eta and Rho_R

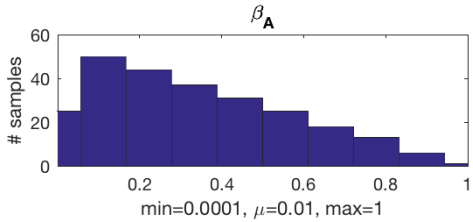


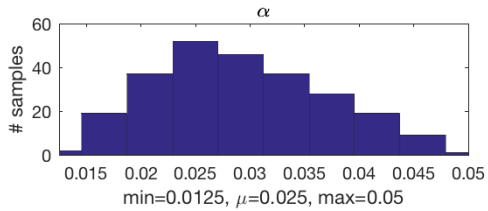
This DOES include eta and Rho_R

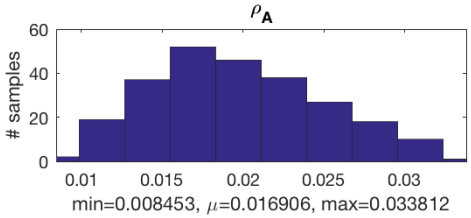


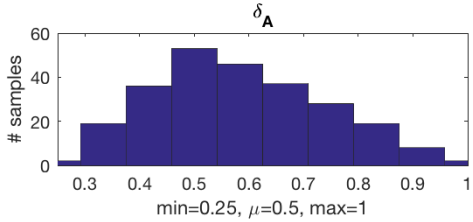
# This DOES include eta and Rho_R

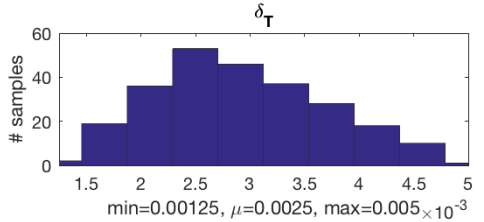


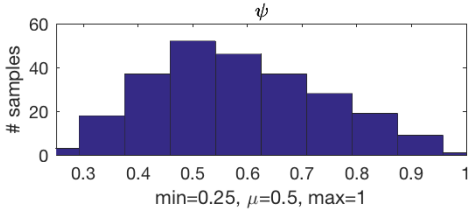



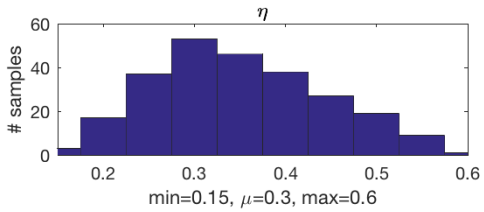



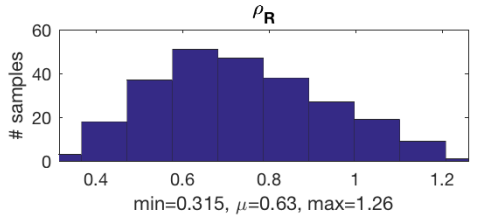



# Patient Data Analysis TCGA Population n=1085 **