A Novel Computational Modeling Framework to Analyze Synovial-Tissue Based Drug Targets and Diagnostic Biomarkers in Rheumatoid Arthritis

Project ID: CBIO021

Paridhi Latawa

Liberal Arts and Science Academy (LASA)

Austin, Texas, USA

Introduction: Rheumatoid Arthritis

- Immune system releases inflammatory chemicals that attack the synovial tissue in joints
- Affects > 20 million people worldwide
- Only 20-30% of patients reach low disease activity even with advanced therapies
- Personal experiences motivated me to pursue project

Clinically Heterogeneous & Cascading Nature of RA

Increased inflammation throughout body	→	**Increased risk of heart disease & attack on pericardium**
Joint pain	→	**Decreased exercise among patients & higher obesity**
Uncontrolled inflammation	→	**Joint deformities, painful bone erosion, weak cartilage**

Within 10 years of onset, **50%** of patients have to discontinue full-time jobs

Intro: Current Treatments & Diagnostics

- Current RA medicines effective, but have severe side effects → create drugs targeting specific RA network
- Early detection has minimized JD & improved patient outcomes [13]
- Need specific diagnostic biomarkers that reflect biological actions in synovial tissue [14]
- Inflamed synovial tissue has potential for drug targeting (enhanced permeability & retention effect)
- Drugs like Amectra, when treated with methotrexate & TNF-i, interfere in JD
- Aim to similarly ID drug targets & biomarkers to alleviate JD & halt RA
- Biological computational modeling
  - Mathematical models in biology bridge understanding of proposed molecular interactions & resultant tissue-level effects
  - Models expressed through sets of ODEs which take discrete time-state measurements & represent how variables evolve over time

Medication Type	Drug Examples	Function	Side Effects
NSAIDs	Ibuprofen (Advil, Motrin IB), naproxen sodium (Aleve), etc.	Reduce inflammation & pain	Stomach irritation, heart problems, kidney damage
DMARDs	Methotrexate, Plaquenil, Azulfidine	Slow progression of RA. Save joints & tissues from permanent damage	Liver damage, severe lung infection
Targeted synthetic DMARDs	Rinvoq, Olumiant	Used if regular DMARDs & biologics aren't effective.	Risk of blood clots in lungs, heart-related events, cancer
Steroids	Corticosteroid Medications (prednisone)	Reduction of inflammation, pain, & joint damage	Bone thinning, weight gain, diabetes
Biological Agents	Humira, Cimzia, Simponi, Rituxan, Actemra	Generally paired with DMARD like methotrexate; Newer class of DMARDs	Increased risk of infection

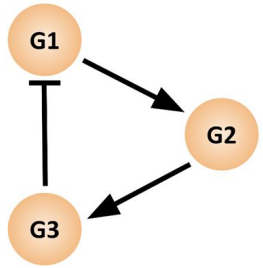
Table 1: *Medication types used to treat Rheumatoid Arthritis (6)*

Research Question, Hypothesis, & Purpose

- **Question**: What genes or proteins are directly correlated to joint destruction in Rheumatoid Arthritis? How can specific drug targets & diagnostic biomarkers better treat and diagnose RA?
- **Purpose**: To identify synovial tissue-based drug targets & diagnostic biomarkers by creating & simulating an *in silico* mathematical model that provides a confirmed diagnosis of RA & assist in developing novel RA treatments
- **Hypothesis**: Based on previous research, targeting VAV1, LCK, & CD4 is important as they are significantly upregulated in patients w/ RA & linked to tissue response/cartilage.

Methods

Fig 1: *3 gene node networks depicting G1, G2, G3 for which example equations are derived (Liu et al)*



Software Libraries:

Google Colaboratory	Python	Numpy library	matplotlib.pyplot functions	Pandas library	seaborn library	label lines function
Platform for all code	Language used for coding	Used to create interval grid of time points of all 25 interactors	Used to plot all interval points of each of the 25 interactors in a graph	Used to create dataframe displaying time series values of 25 interactors	Used to create heatmap of time series values of all 25 interactors	Used to label lines of figures

Ordinary Differential Equations (ODEs):

- Constructed ODEs for 30 gene networks
- Referenced textbook by Markus Covert
- Process of writing equations:
  - Derived base & activator equations
  - Added degradation, dilution, transcription, & interaction constants
  - Wrote specialized equations for each 2 & 3 gene network
  - Combined equations for interactors w/ respect to entire system
  - Applied assumptions to simplify ODEs

Differential Equation Breakdown:

General Form of DE: $\frac{dx}{dt} = \sum Rate_{Production} - Rate_{Loss}$

General DE for mRNA transcript: $\frac{d[mRNA]}{dt} = Rate_{Transcription} - Rate_{Decay} - Rate_{Dilution}$

Specific DE for mRNA transcript: $\frac{d[mRNA]}{dt} = f(G_i[t]) + k_{Transcription} - k_{Degradation} * mRNA[t]$

General DE for small molecules: $\frac{d[mol]}{dt} = k_{transcription} - k_{dilution} * mol[t]$

Specific DE for small molecules: $\frac{d[mol]}{dt} = f(G_i[t]) + k_{transcription} - k_{dilution} * mol[t]$

Base Activator DE: $\frac{d(G1)}{dt} = \frac{k_{1_3}}{1 + k_{1_3} * G3[i]} - k_{deg} * G1[i] + k_{transcription}$

Base Inhibitor DE: $\frac{d(G2)}{dt} = \frac{k_{2_1} * G1[i]}{1 + k_{2_1} * G1[i]} - k_{deg} * G2[i] + k_{transcription}$

Derived Equation for REV-ERB: $\frac{d(G4)}{dt} = \frac{k_{4_2}}{1 + k_{4_2} * G2[i]} * G2[i] + \frac{k_{4_3}}{1 + k_{4_3} * G3[i]} - k_{deg} * G4[i] + k_{transcription}$

Methods

Baseline Model:

- Based on research, 9 pathways of interest added to create model
- Baseline RA model simulated elevated conditions
- 25 molecules and 30 2 & 3 gene network interactions incorporated
- Δ in concentration measured over time
- Output:
  - Graph of RA model
  - Heat maps of final concentration/expression

Interaction Pathway	Scientific Study/Source
a. ROR → Th-17 → IL-17 → JD b. REV-ERB -| Th-17 → IL-17 → JD	Chang et al (23), Amir et al (24), Robert & Miossec (25)
LCK → FOXP3	Nakahira et al (26)
Thymocyte → LCK → CD4 T-cells (bonafide Treg cells) → FOXP3 → RA	Li et al (27)
LCK → VAV1	Han et al (28)
VAV1 → CD4 (CD4+ T cells) → Inflammatory Cytokines	Kassem et al (30)
ZAP70 → Cry1	Lewintre et al (31, 32)
CD4 – (macrophage) → TNF-α	Castro-Sanchez and Roda-Navarro (33)
CD4+ T cell -- (cell differentiation) → Th-17	Tesmer et al (34)

Table 2: *Table presenting rationales for the added pathways.*

Perturbations:

- Drug targets & diagnostic biomarkers were ID, by altering interaction dynamics
- 92 perturbations on 46 constants incorporated
- 2 possible perturbations for each interaction constant = 100 or 0.01
- Recorded perturbations with significant fluctuations in magnitude fold change or fractional difference

Data & Results

26 quantities graphed from initial log growth to homeostasis

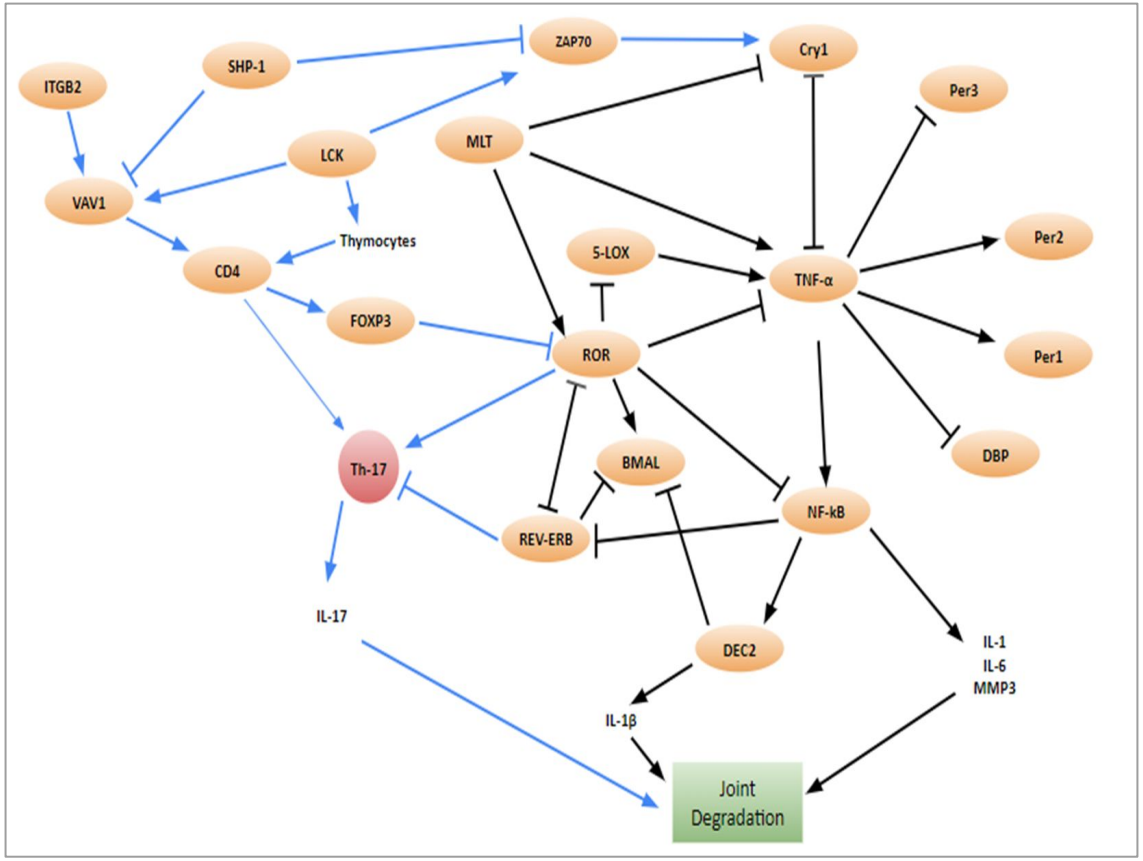


Figure 2: *Adjusted RA model. Added interactions are in blue. Arrow represents activation & "T" represents inhibition. Orange circles are genes. Components in no boxes or red are small molecules.*

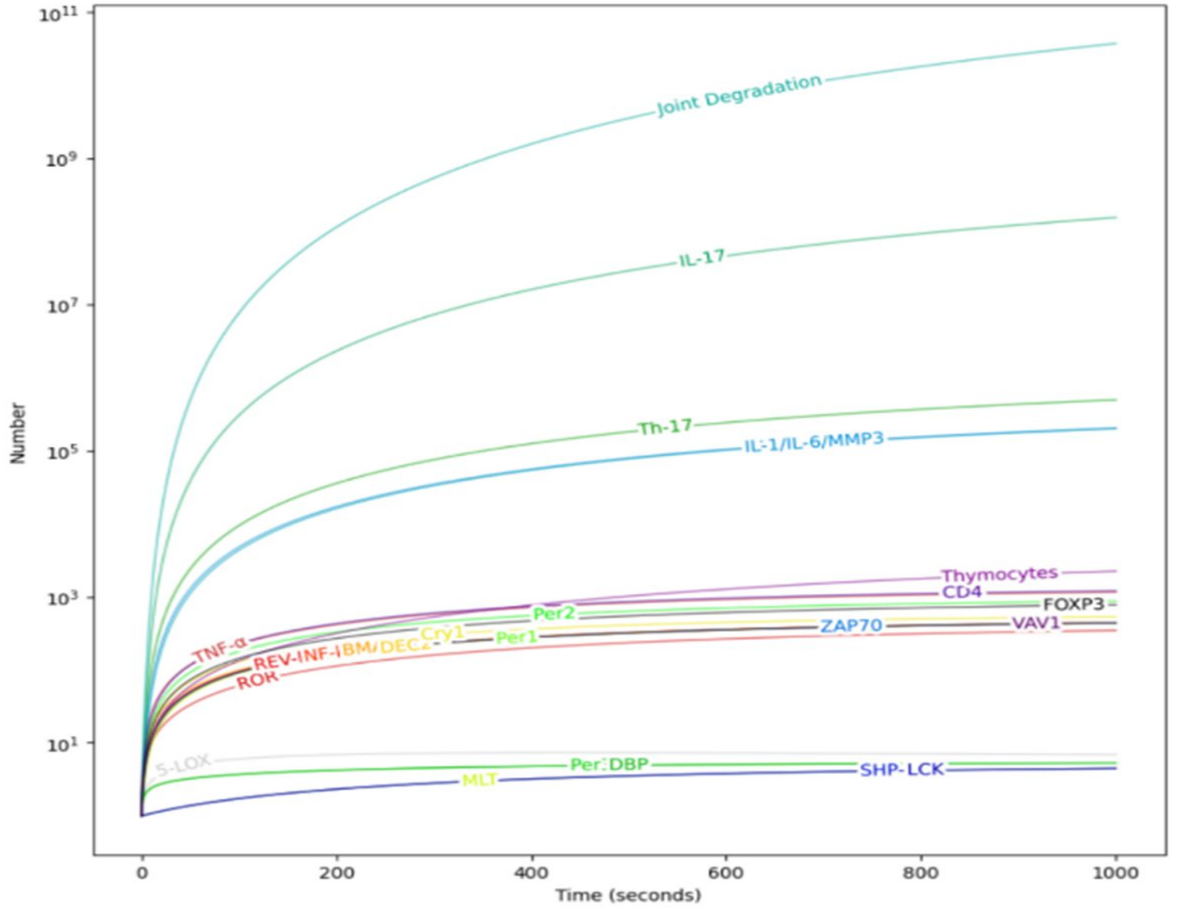


Figure 3: *Graph of change in qty/expression of molecules, genes, & phenomena. x-axis is time & y-axis is count of change on log scale.*

Ssome perturbations caused fold changes in a similar range as JD; others with significant difference were recorded

Pathway	Original Constant for Pathway	Perturbed Constant for Pathway	Change in Joint Degradation at time t = 4950
VAV1 activating CD4	K_22_24 = 1	K_22_24 = 0.01	9.984670e+11
MLT activating ROR	K_3_9 = 1	K_3_9 = 0.01	7.461039e+11
ROR activating Th-17	K_14_3 = 1	K_14_3 = 0.01	7.400516e+11
Th-17 activating IL-17	K_15_14 = 1	K_15_14 = 0.01	1.167708e+10
LCK activating CD4	K_22_21 = 1	K_22_21 = 0.01	7.606287e+11
Thymocytes activating CD4	K_22_23 = 1	K_22_23 = 0.01	1.055373e+12
VAV1 activating CD4	K_22_24 = 1	K_22_24 = 0.01	9.984670e+11
LCK activating Thymocytes	K_23_21 = 1	K_23_21 = 0.01	1.061217e+12

Table 3: *Perturbations that reduced JD. Red indicates perturbation is upregulating gene & green indicates that perturbation is downregulating gene. t = 4950 is homeostasis.*

Pathway	Original Constant for Pathway	Perturbed Constant for Pathway	Change in Joint Degradation at time t = 4950
MLT activating ROR	K_3_9 = 1	K_3_9 = 100	1.148312e+12
TNF-α activating REV-ERB	K_4_2 = 1	K_4_2 = 0.01	1.109264e+12
ROR activating Th-17	K_14_3 = 1	K_14_3 = 100	3.453803e+13
REV-ERB inhibiting Th-17	K_14_4 = 1	K_14_4 = 0.01	1.481887e+12
CD4 activating Th-17	K_14_22 = 1	K_14_22 = 100	1.146315e+14
Th-17 activating IL-17	K_15_14 = 1	K_15_14 = 100	1.073755e+14
DEC2 activating IL1β	K_17_7 = 1	K_17_7 = 100	1.121071e+12
NF-kB activation IL-1/IL-6/MMP3	K_18_5 = 1	K_18_5 = 100	1.121270e+12
LCK activating CD4	K_22_21 = 1	K_22_21 = 100	1.147978e+12

Table 4: *Perturbations that increased level of joint degradation. Similar setup to Table 3.*

Potential Drug Targets–reduced JD:

- Inhibition of VAV1-CD4
- Inhibition of LCK-Thymocyte
- Inhibition of MLT-ROR
- Inhibition of ROR-Th 17
- Inhibition of Th-17-IL-17
- Inhibition of LCK-CD4

Diagnostic Biomarkers- increased JD:

- Activation of MLT-ROR
- Activation of DEC2-IL1□
- Activation of NF-kB-interleukin
- Activation of LCK-CD4, pathways
- Inhibition of NF-α-REV-ERB
- Inhibition of REV-ERB-Th-17

ODEs used to create computational model & output heatmap depicting concentration over time

Label	Molecule Name	Corresponding Differential Equation
G1	5-LOX	$\frac{d(G1)}{dt} = \frac{k_{13}}{1 + k_{13} * G3[i]} - k_{deg} * G1[i] + k_{transcription}$
G2	TNF-α	$\frac{d(G2)}{dt} = \frac{k_{21}}{1 + k_{21} * G1[i]} * G1[i] + \frac{k_{23}}{1 + k_{23} * G3[i]} + \frac{k_{28}}{1 + k_{28} * G8[i]} + \frac{k_{29}}{1 + k_{29} * G9[i]} * G9[i] + \frac{k_{222}}{1 + k_{222} * G22[i]} * G22[i] - k_{deg} * G2[i] + k_{transcription}$
G3	ROR	$\frac{d(G3)}{dt} = \frac{k_{34}}{1 + k_{34} * G4[i]} + \frac{k_{39}}{1 + k_{39} * G9[i]} * G9[i] + \frac{k_{325}}{1 + k_{325} * G25[i]} - k_{deg} * G3[i] + k_{transcription}$
G4	REV-ERB	$\frac{d(G4)}{dt} = \frac{k_{42}}{1 + k_{42} * G2[i]} * G2[i] + \frac{k_{43}}{1 + k_{43} * G3[i]} - k_{deg} * G4[i] + k_{transcription}$
G5	NF-kB	$\frac{d(G5)}{dt} = \frac{k_{52}}{1 + k_{52} * G2[i]} * G2[i] + \frac{k_{53}}{1 + k_{53} * G3[i]} - k_{deg} * G5[i] + k_{transcription}$
G6	BMAL	$\frac{d(G6)}{dt} = \frac{k_{63}}{1 + k_{63} * G3[i]} * G3[i] + \frac{k_{64}}{1 + k_{64} * G4[i]} + \frac{k_{65}}{1 + k_{65} * G5[i]} + \frac{k_{67}}{1 + k_{67} * G7[i]} - k_{deg} * G6[i] + k_{transcription}$
G7	DEC2	$\frac{d(G7)}{dt} = \frac{k_{75}}{1 + k_{75} * G5[i]} * G5[i] - k_{deg} * G7[i] + k_{transcription}$
G8	Cry1	$\frac{d(G8)}{dt} = \frac{k_{82}}{1 + k_{82} * G2[i]} + \frac{k_{89}}{1 + k_{89} * G9[i]} + \frac{k_{819}}{1 + k_{819} * G19[i]} * G19[i] - k_{deg} * G8[i] + k_{transcription}$
G9	MLT	$\frac{d(G9)}{dt} = 0 - k_{deg} * G9[i] + k_{transcription}$
G10	Per1	$\frac{d(G10)}{dt} = \frac{k_{102}}{1 + k_{102} * G2[i]} * G2[i] - k_{deg} * G10[i] + k_{transcription}$
G11	Per2	$\frac{d(G11)}{dt} = \frac{k_{112}}{1 + k_{112} * G2[i]} * G2[i] + \frac{k_{118}}{1 + k_{118} * G8[i]} * G8[i] - k_{deg} * G11[i] + k_{transcription}$

Table 5: *Combined ODEs for 11 molecules, gene, or processes. Rest In lab notebook*

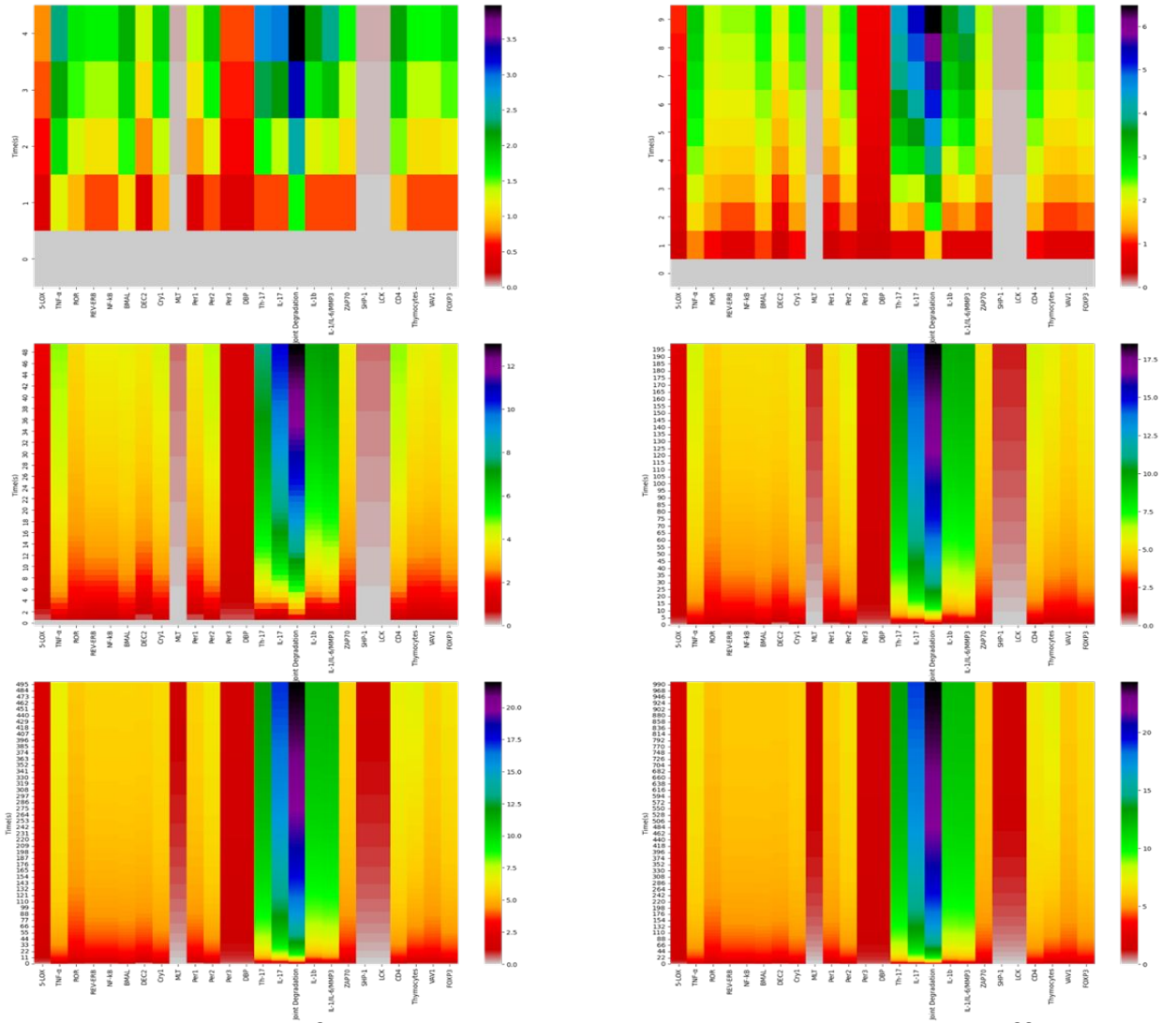


Fig 4: *Heat maps of expression over 5000 sec. Each represents different time scale. Color progresses from red (lower expression) to purple (high expression).*

Discussion

- RA is one of the most prevalent chronic inflammatory diseases
- ID more specific drug targets & diagnostic biomarkers is crucial to alleviate RA

Strengths

- Model incorporated variety of genes, proteins, & small molecules via specialized differential equations → more accurate results
- Easily track measurements at any time → precise simulation
- Model uses well-developed basic framework & adds scientifically-based proposed interactions to accurately simulate specific network that has never been studied *in silico* previously
- All possible perturbations were tested in model, allowing ID of most promising drug targets for RA

Limitations

- Degradation & dilution constants derived based on assumptions of properties of molecules of interest
- While model assumed translation is happening at slower rate than transcription such that transcription is bottleneck, if a certain gene does not follow assumption where translation is faster than transcription, model would have to be altered
- “1” used as comparative value of concentrations & constants
- Results should be confirmed in vitro

Key Findings

- Created framework with potential to find drug targets & diagnostic biomarkers
- Inhibition of LCK-CD4, VAV1-CD4, & MLT-ROR pathways could serve as drug targets
- Increased activity of DEC2-IL1□, & NF-kB interleukin pathway & decreased activity of TNF-α-REV-ERB pathway could serve as diagnostic biomarkers
- These are novel findings with potential implications in therapeutic development & disease diagnosis
- Significance of targeting pathways directly related to joint destruction to diagnose or inhibit RA
- Greater confidence on interactions in RA
- Illustration of powerful role computational modeling in understanding RA & other diseases

Further Research

- Ensuring concentration/constants are biologically correct
- Changing constants from theoretical value of 1 to molecule specific values
- **Run:**
  - RNA sequencing: measure # of each transcript in a cell at specific time intervals
  - Single-cell sequencing: measure cell's RNA transcript concentration & find initial concentrations
  - Binding assays for proteins: measure protein binding strength for interaction constants
- Find molecular weights of each gene transcript to improve degradation constants
- Conduct AI-based analysis to to understand interactions
- Ultimately develop therapeutics & diagnostic tools for RA

References

Figures are my own or are created from cited sources

1. *"Rheumatoid Arthritis (RA)." Centers for Disease Control and Prevention, Centers for Disease Control and Prevention, 27 July 2020, www.cdc.gov/arthritis/basics/rheumatoid-arthritis.html.*
2. *"Rheumatoid Arthritis." Mayo Clinic, Mayo Foundation for Medical Education and Research, 18 May 2021, www.mayoclinic.org/diseases-conditions/rheumatoid-arthritis/diagnosis-treatment/drc-20353653#:~:text=There is no cure for, modifying antirheumatic drugs (DMARDs).*
3. *Tomlin, Claire J., and Jeffrey D. Axelrod. "Biology by Numbers: Mathematical Modelling in Developmental Biology." Nature Reviews Genetics, vol. 8, no. 5, 2007, pp. 331–340., doi:10.1038/nrg2098.*
4. *"Mathematical Models in Biology - WebHome Kuttler/ · Chapter 1 Introduction to Modelling Literature:" Dokumen.tips, dokumen.tips/documents/mathematical-models-in-biology-webhome-kuttler-chapter-1-introduction-to.html.*
5. *Daun, Silvia, et al. "Equation-Based Models of Dynamic Biological Systems." Journal of Critical Care, vol. 23, no. 4, 2008, pp. 585–594., doi:10.1016/j.jcrc.2008.02.003.*
6. *Google Colaboratory, Google, colab.research.google.com/notebooks/intro.ipynb?utm_source=scs-index.*
7. *Covert, Markus W.,Fundamentals of Systems Biology: from Synthetic Circuits to Whole-Cell Models. CRC Press, 2017.*
8. *Liu, Enze, et al. "Gene Regulatory Network Review." Encyclopedia of Bioinformatics and Computational Biology, 2019, pp. 155–164., doi:10.1016/b978-0-12-809633-8.20218-5.*
9. *Philips, Ron Milo & Ron. ""How Fast Do RNAs and Proteins Degrade?" Cell Biology by the Numbers How Fast Do RNAs and Proteins Degrade Comments, book.bionumbers.org/how-fast-do-rnas-and-proteins-degrade/.*
10. *Chang, Christina, et al. "The Nuclear Receptor REV-ERBα Modulates Th17 Cell-Mediated Autoimmune Disease." Proceedings of the National Academy of Sciences, vol. 116, no. 37, 2019, pp. 18528–18536., doi:10.1073/pnas.1907563116.*
11. *Amir, Mohammed, et al. "REV-ERBα Regulates TH17 Cell Development and Autoimmunity." Cell Reports, vol. 25, no. 13, 2018, doi:10.1016/j.celrep.2018.11.101.*
12. *Robert, Marie, and Pierre Miossec. "IL-17 in Rheumatoid Arthritis and Precision Medicine: From Synovitis Expression to Circulating Bioactive Levels." Frontiers in Medicine, Google Colaboratory, Google, colab.research.google.com/notebooks/intro.ipynb?utm_source=scs-index.*
13. *Castro-Sánchez, Patricia, and Pedro Roda-Navarro. "Physiology and Pathology of Autoimmune Diseases: Role of CD4 T Cells in Rheumatoid Arthritis." Physiology and Pathology of Immunology, 2017, doi:10.5772/intechopen.70239.*
14. *Radner, Helga, and Daniel Aletaha. "Anti-TNF in Rheumatoid Arthritis: an Overview." Wiener Medizinische Wochenschrift, vol. 165, no. 1-2, 2015, pp. 3–9., doi:10.1007/s10354-015-0344-y.*