Mathematical models of targeted cancer therapies and drug resistance

1. Treatment of chronic lymphocytic leukemia with targeted drugs, and the evolution of drug resistance

2. Spatial dynamics of virus spread, and the treatment of cancers with onlcolytic viruses – determinants of cellular resistance

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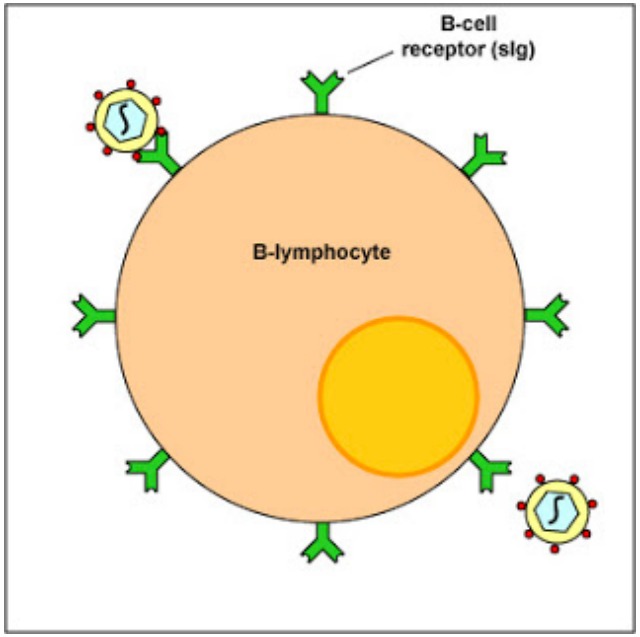
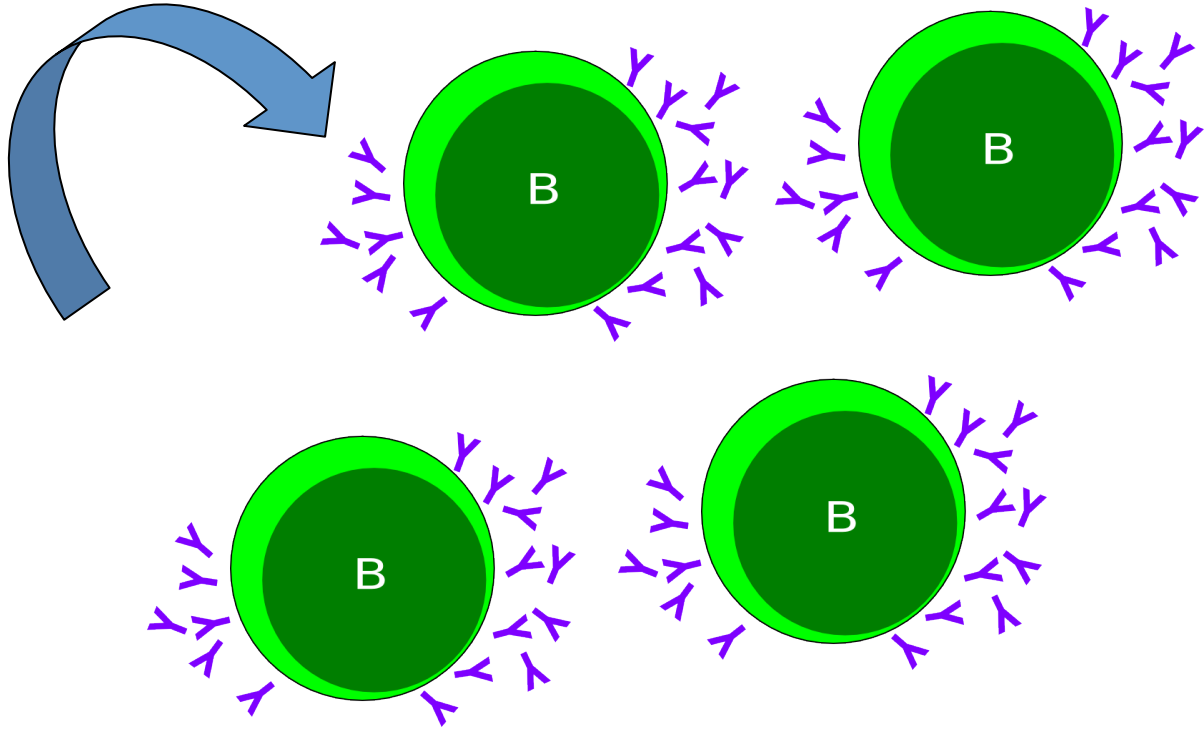
Chronic Lymphocytic Leukemia (CLL)

- most common type of leukemia
- accumulation of small B lymphocytes with mature appearance
- most patients are diagnosed without symptoms during routine blood tests
- Upon diagnosis, a "wait and see" approach is followed.

Cells of origin

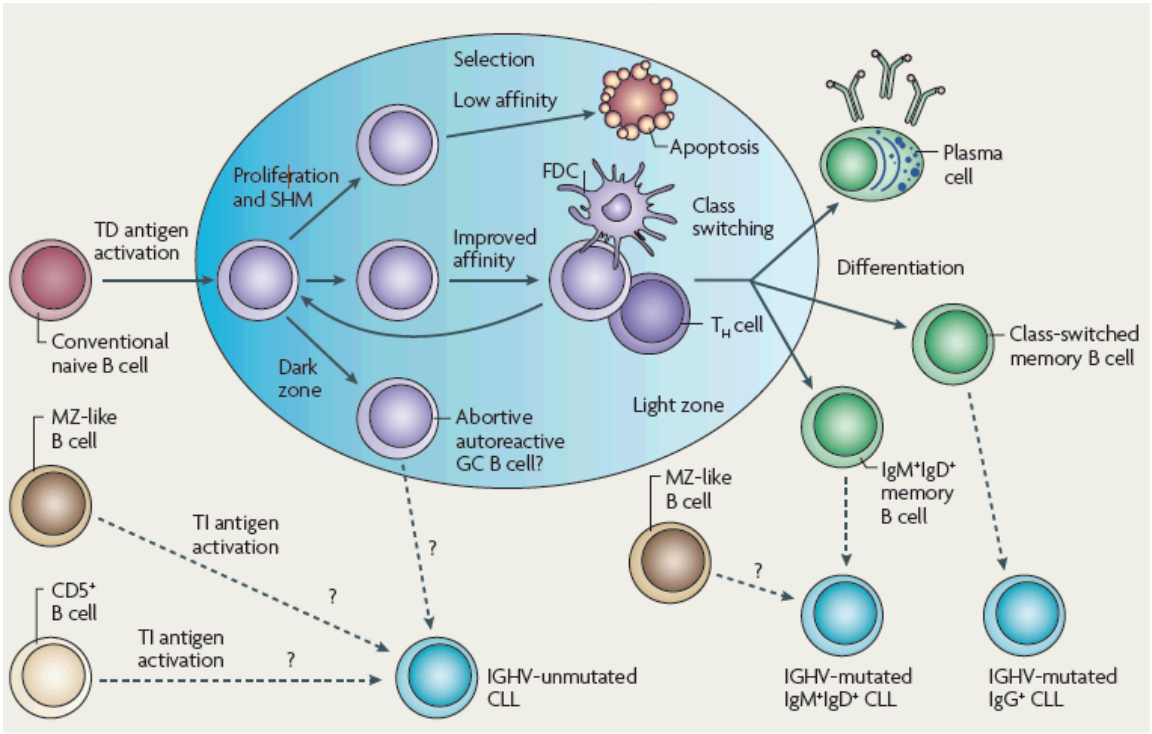


Resting B cell becomes activated by pathogen



Activated B cell proliferates and secretes antibody

Risk factors and heterogeneity



"unmutated"
worse prognosis

"mutated"
better prognosis

Risk factors and heterogeneity

del 13q: Deletion of long arm of chromosome 13, is the most common abnormality (50%). Best prognosis, some never need treatment

Trisomy 12: 20-25% of patients, have intermediate prognosis

del 11q: Deletion of long arm of chromosome 11, relatively poor prognosis, because deletion targets the ATM gene. Occurs in 5-10% of cases

del 17p: deletion of part of short arm of chromosome 17. Poorest prognosis because it inactivates p53. (5-10% of cases)

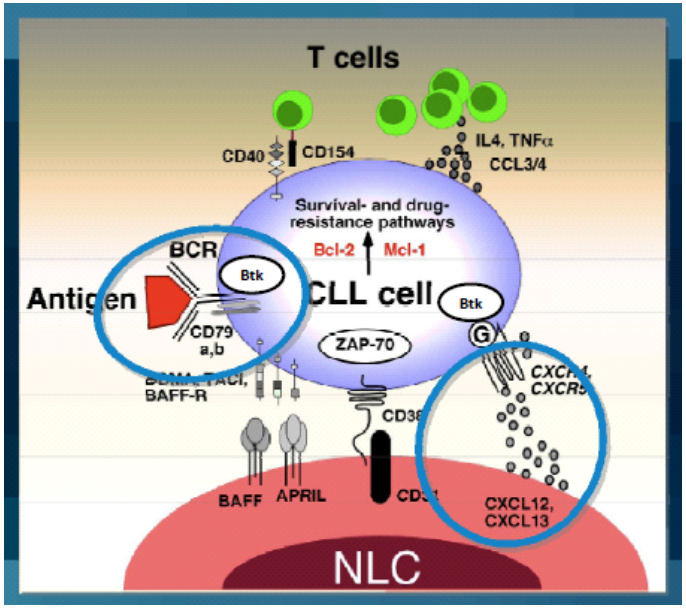
Therapy

up to 2014, the standard was “chemo-immunotherapy” ineffective against more virulent cases, e.g. del 17p or unmutated CLL

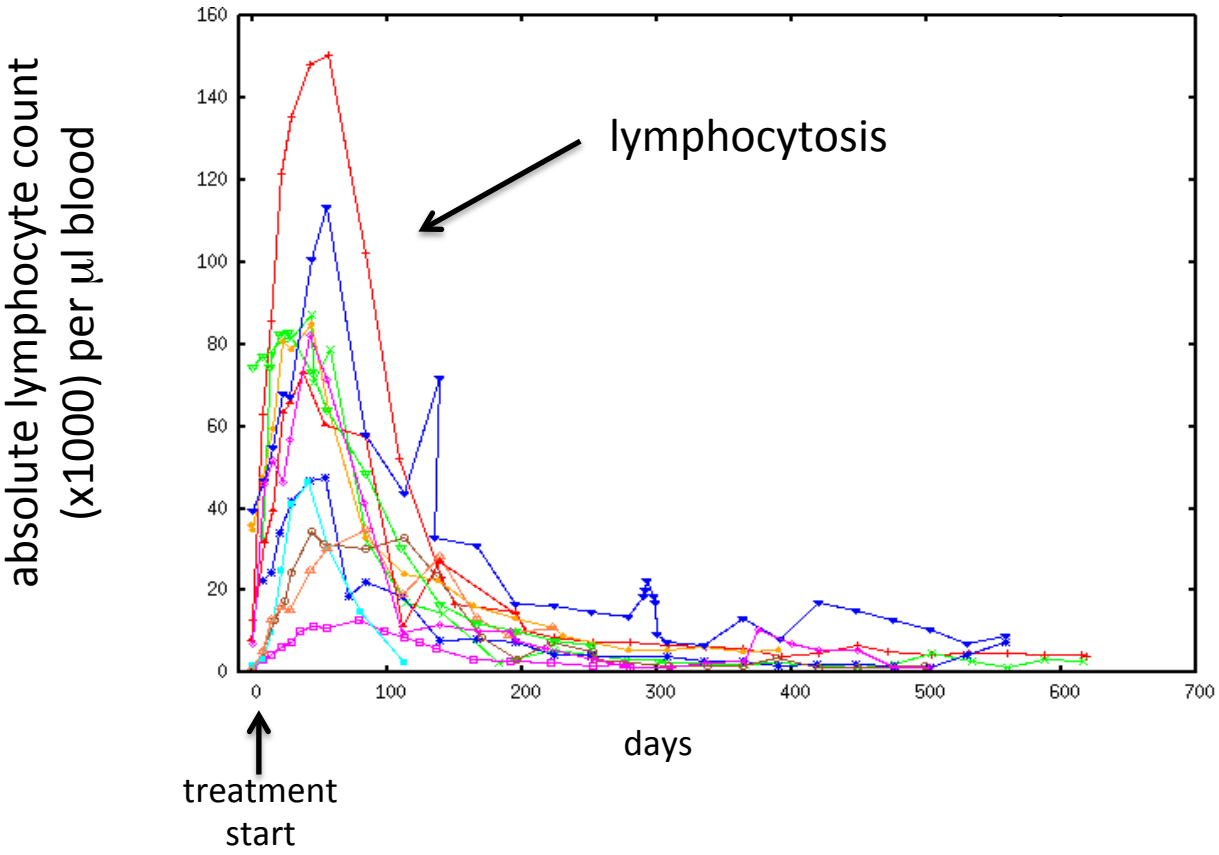
Targeted treatment approaches have emerged.

Ibrutinib

- First Bruton tyrosine kinase (BTK) inhibitor
- acts via specific binding to a cysteine residue in the BTK kinase domain
- inhibits BTK phosphorylation and its enzymatic activity
- Clinically active through:
  - induction of cell death
  - inhibition of proliferation
  - inhibition of tissue homing

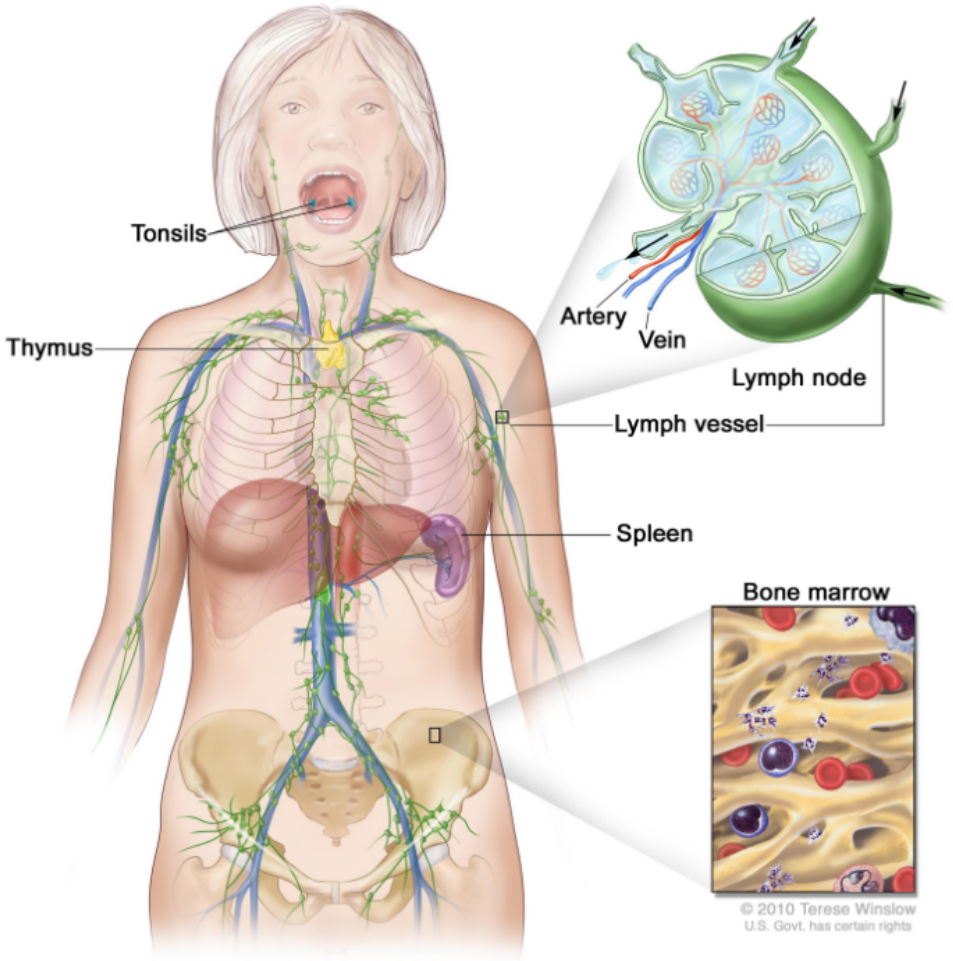


CLL response to Ibrutinib **in previously treated patients** (treatment start at day 0)

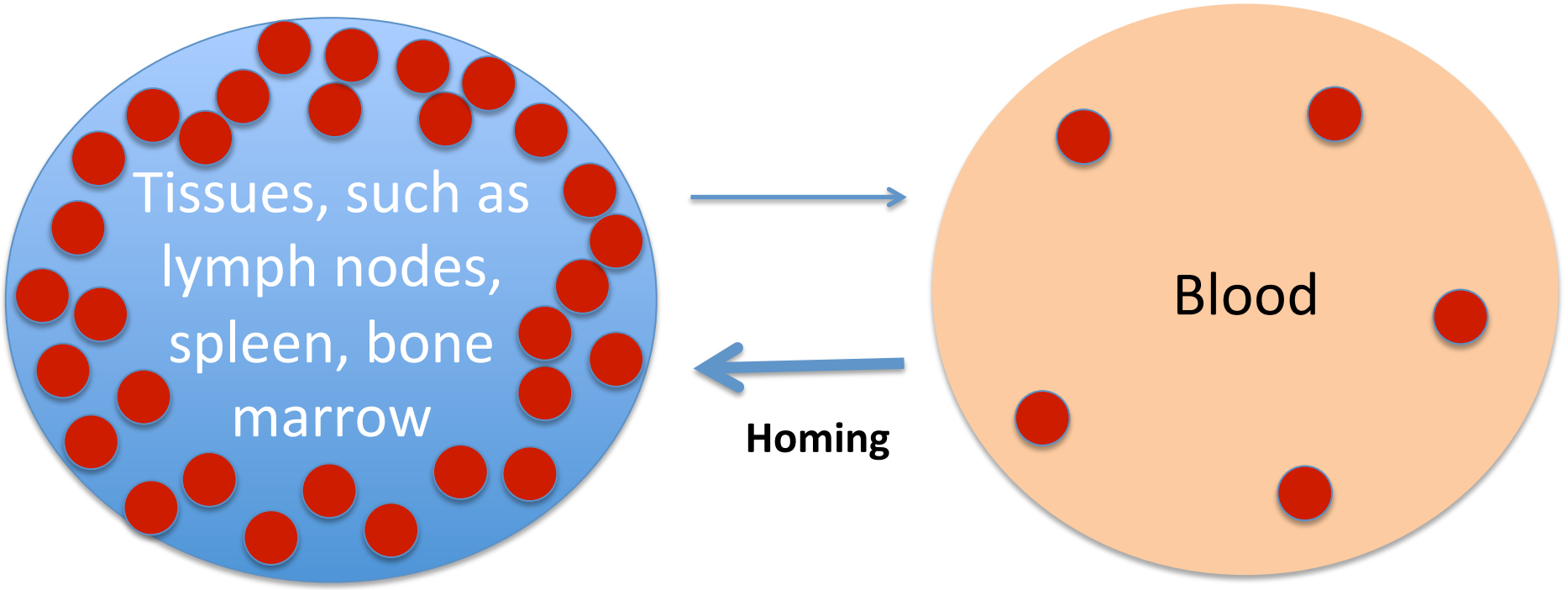


Every patient shows a temporary phase of **lymphocytosis**, where the number of CLL cells in blood increases up to a peak, before eventually declining.

Reasons for lymphocytosis: Compartments



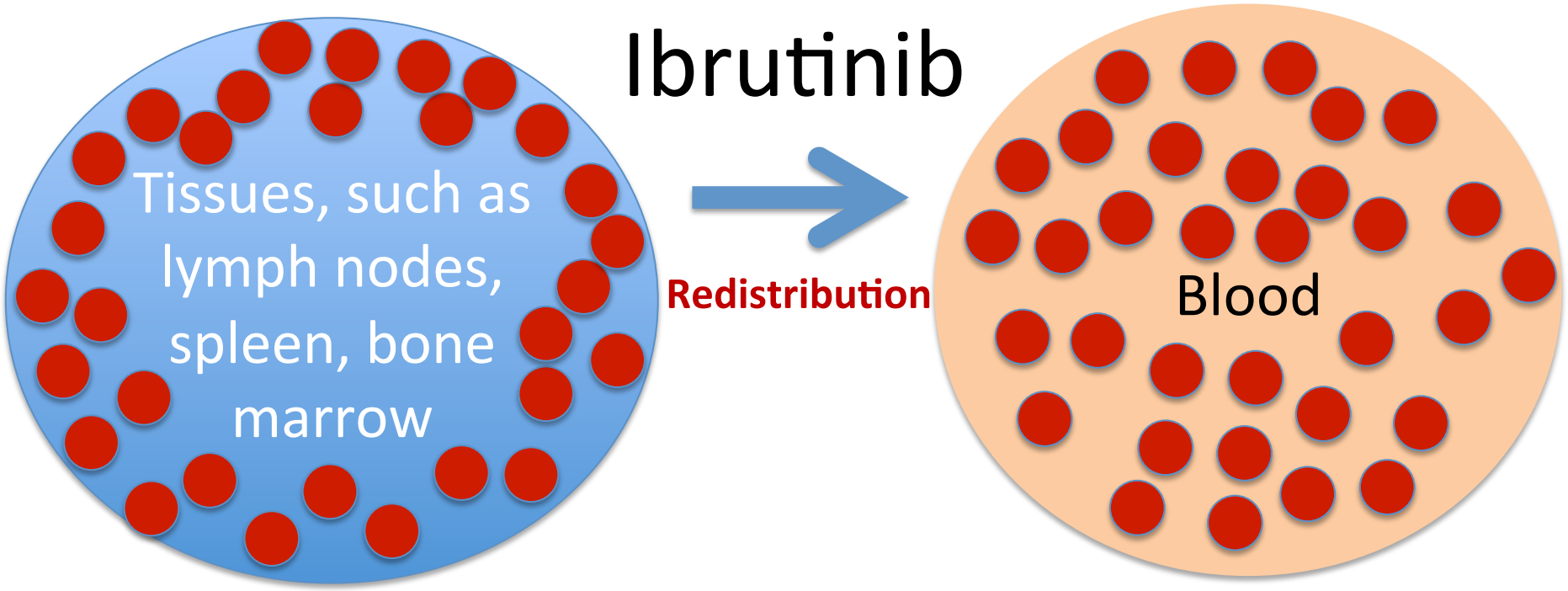
Reasons for lymphocytosis: Compartments



Division and growth, most cells here
=>Microenvironment

No division and growth small fraction of tumor

Reasons for lymphocytosis: Compartments

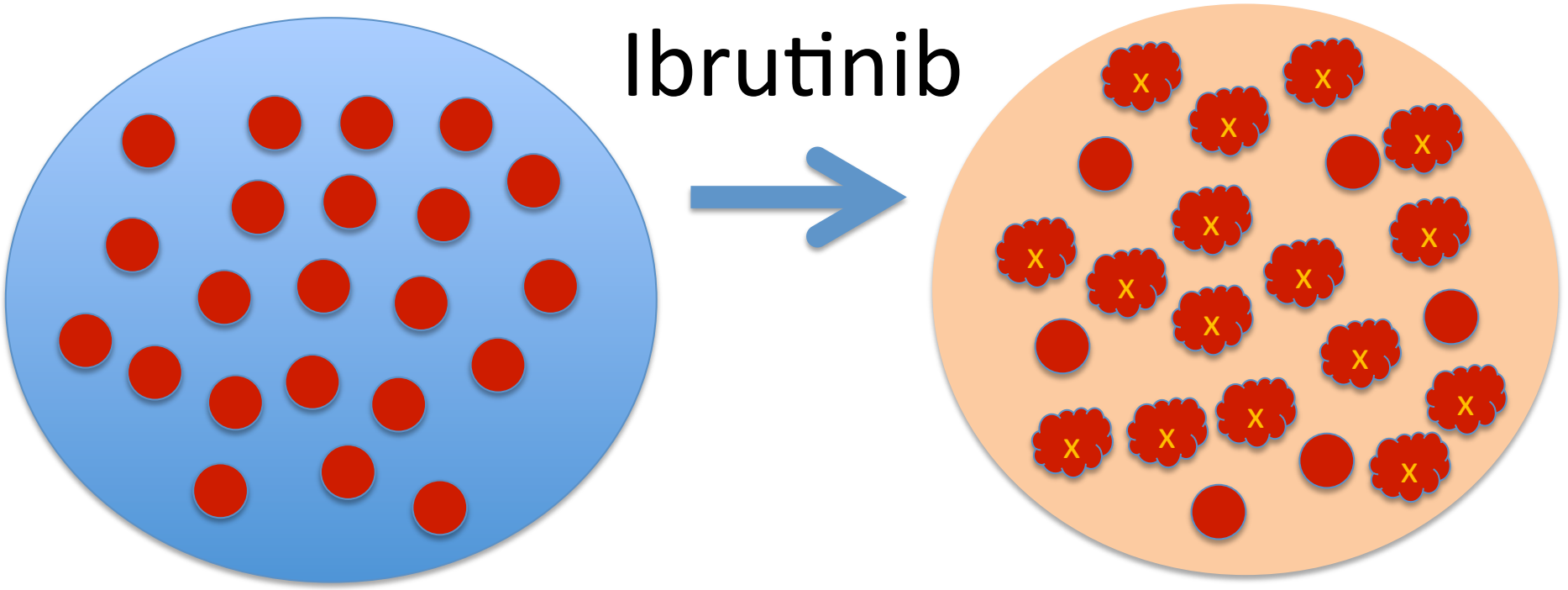


Division and growth, most cells here
=>Microenvironment

No division and growth small fraction of tumor

2 possible scenarios for how ibrutinib affects cells in those compartments

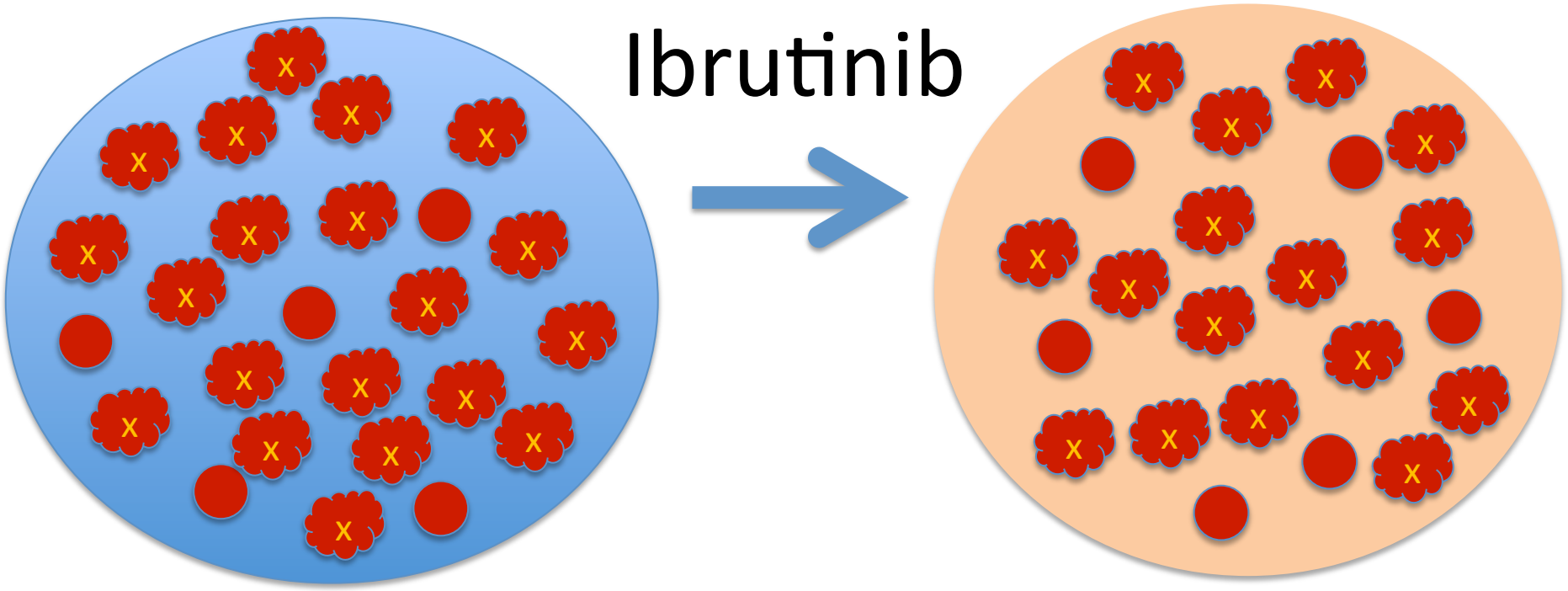
Scenario 1: death “by neglect” in blood



no cell death in tissue

cells die in blood after redistribution, due to lack of microenvironment

Scenario 1: significant cell death in tissue



significant cell death
in tissue

blood
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Kinetics of chronic lymphocytic leukemia (CLL) cells in tissues and blood during therapy with the BTK inhibitor ibrutinib

Dominik Wodarz, Naveen Garg, Natalia L. Komarova, Ohad Benjamini, Michael J. Keating, William G. Wierda, Hagop Kantarjian, Danelle James, Susan O'Brien and Jan A. Burger

What does this lymphocytosis mean?

- Drug simply causes cells to shift compartment
- This lead to "death by neglect" in blood

=> Less effective drug

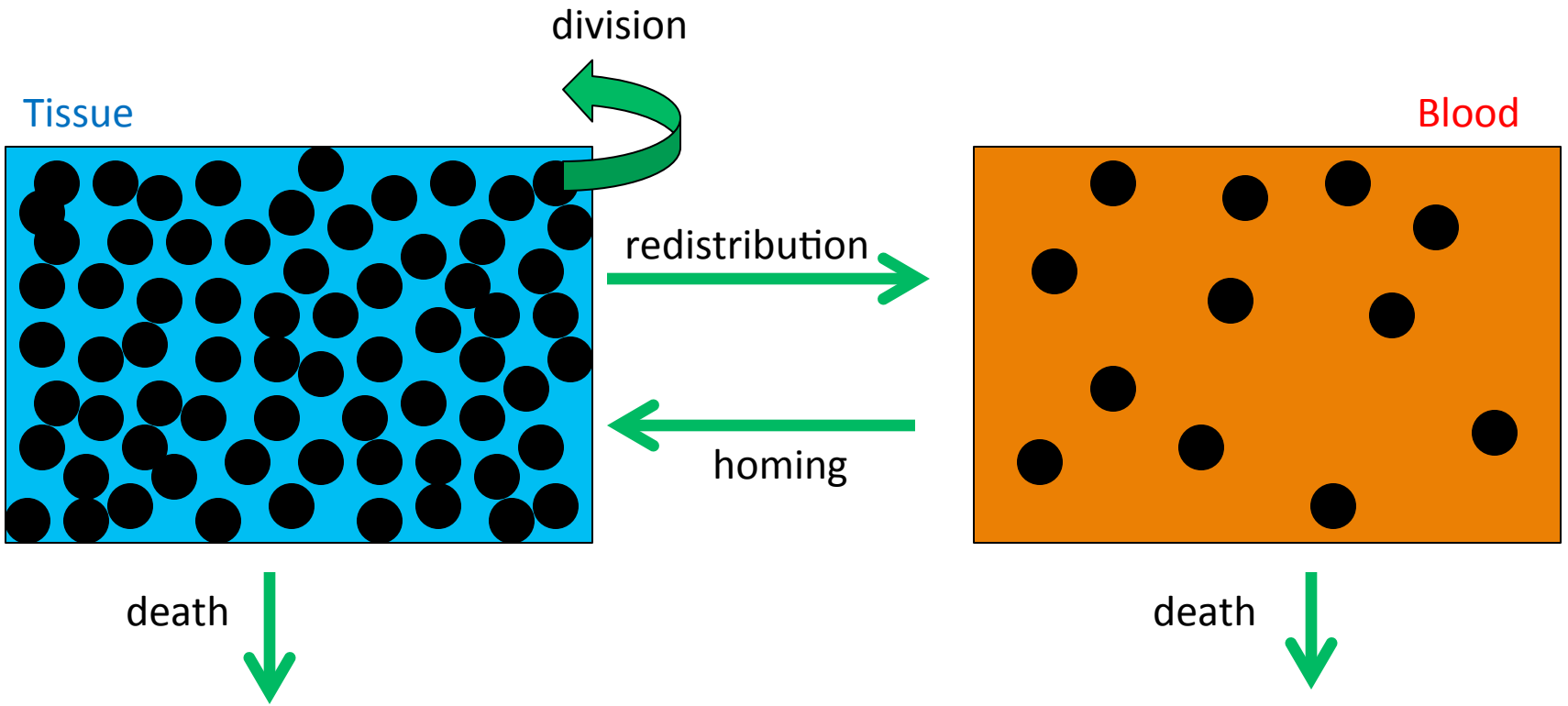
Drug causes significant cell death in tissue
Only a minority of tumor redistributes to blood

=> More effective drug

To answer question:
apply mathematical models to clinical data in order to measure kinetic parameters

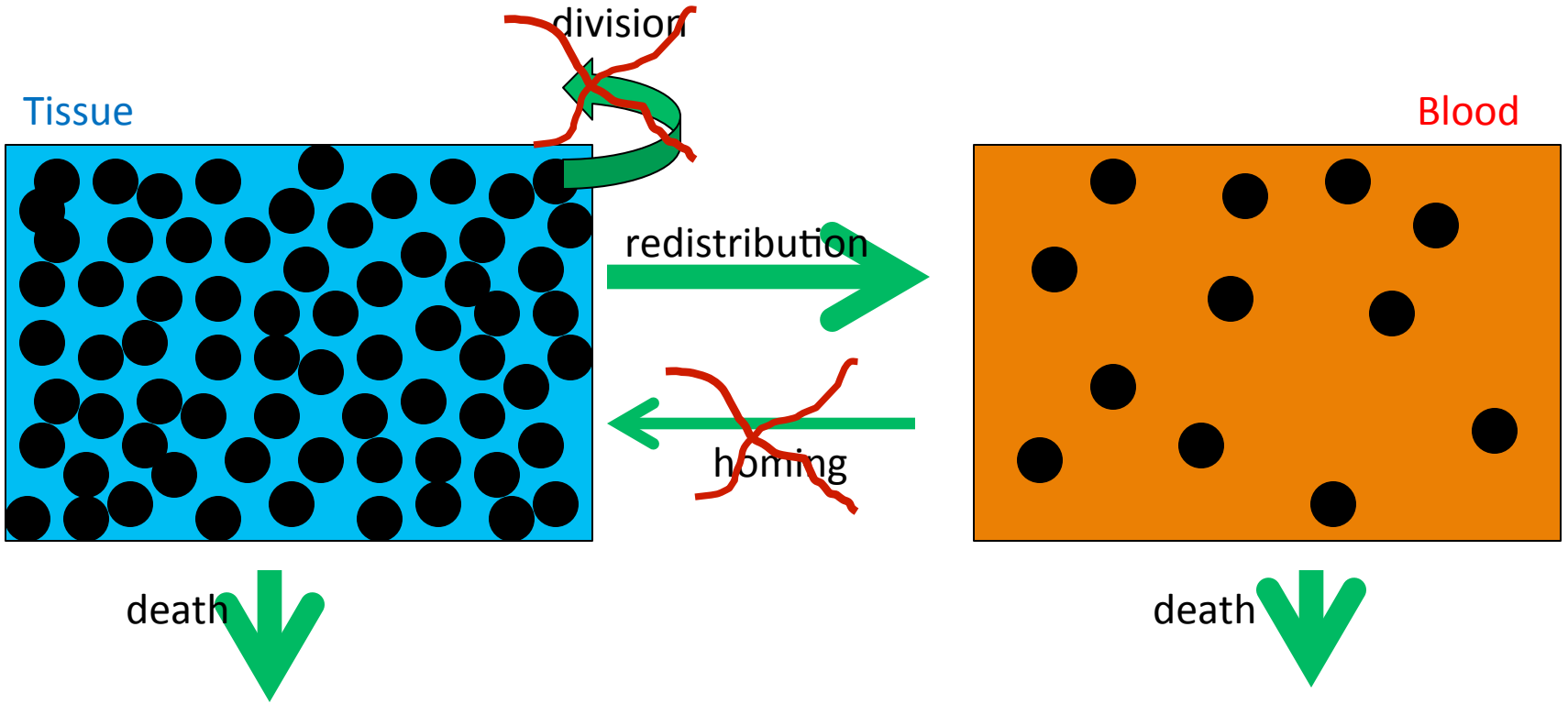
Mathematical model

We considered a two-compartment model for CLL dynamics:



Mathematical model

Treatment:



Mathematical model

m = rate of redistribution

d_1= CLL cell death rate in tissue

d_2 = CLL cell death rate in blood

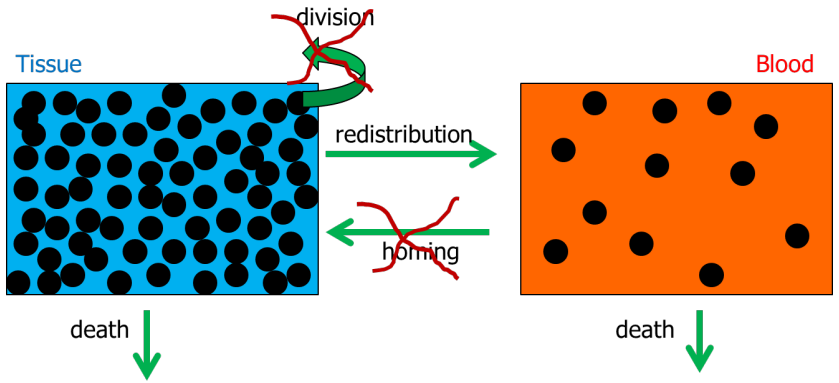
c = factor to account for the observation that CLL cells stabilize at low levels in the long term

nodal response rate: $\alpha = m + d_1$

$$\frac{dx}{dt} = -mx - d_1(x - c)$$

$$\frac{dy}{dt} = mx - d_2 y$$

Treatment:



idea: fit model to treatment data and estimate the parameters

Model

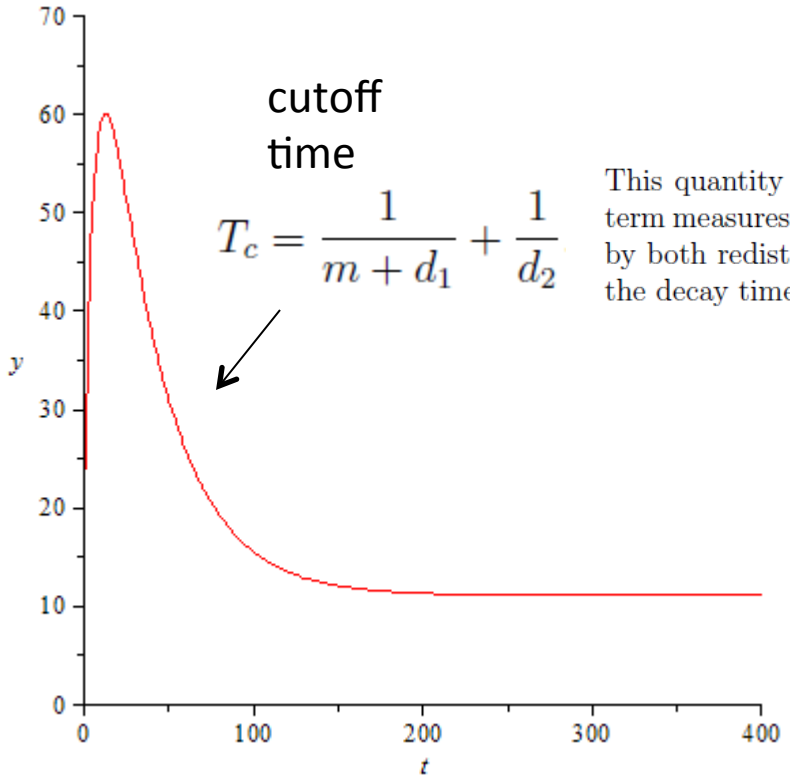
Aims:

- estimate crucial parameters
- calculate the percentage of pre-treatment tissue tumor burden that redistributes into the blood

Model

Relative number of cells redistributed from tissue to blood:

$$Z(t) = \frac{\int_0^t mx(t')\,dt'}{x_0} = \frac{m}{\alpha x_0}\left((x_0 - C_x)(1 - e^{-\alpha t}) + \alpha C_x t\right). \qquad (11)$$



cutoff time

$$T_c = \frac{1}{m + d_1} + \frac{1}{d_2}$$

This quantity is a composite of two characteristic times of decay: the first term measures the decay-time of CLL lymphocytes in tissues (and it is defined by both redistribution and death processes), and the second term measures the decay time in blood, defined uniquely by the death rate d_2.

Tumor stabilizes due to parameter *c*

this phase is not interesting. Here *Z* grows linearly in time because of remaining equilibirum level of CLL cells in tissue

Model fitting

Model contains 2 variables:

cells in tissues

$$\frac{dx}{dt} = -mx - d_1(x - c)$$

cells in blood => absolute lymphocyte counts

$$\frac{dy}{dt} = mx - d_2 y$$

Model fitting

Model contains 2 variables:

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$$\frac{dx}{dt} = -mx - d_1(x - c)$$

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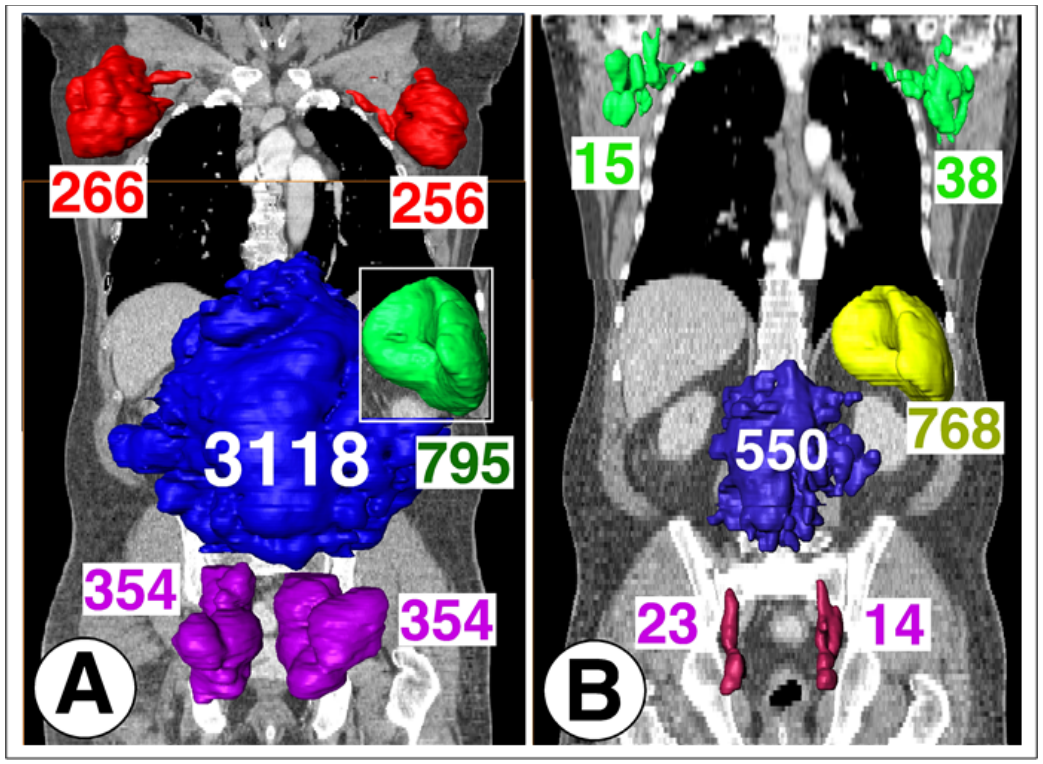
$$\frac{dy}{dt} = mx - d_2 y$$

Volumetric Analysis of tissue tumor burden



Volumetric analyses of CLL lymph node and spleen manifestation (A) before and (B) during therapy with ibrutinib.

Depicted are CT images from a representative CLL patient from our series with superimposed reconstruction of main areas of CLL involvement, highlighted in color. The volumes of the axillary (red), intra-abdominal (blue), inguinal (purple) and spleen (green, yellow) disease manifestations are displayed next to each involved area.

Volumetric analysis done for 3 time points: one before treatment, two during treatment

(bone marrow burden difficult to measure => parameter estimates are lower bounds)

Model fitting

Model contains 2 variables:

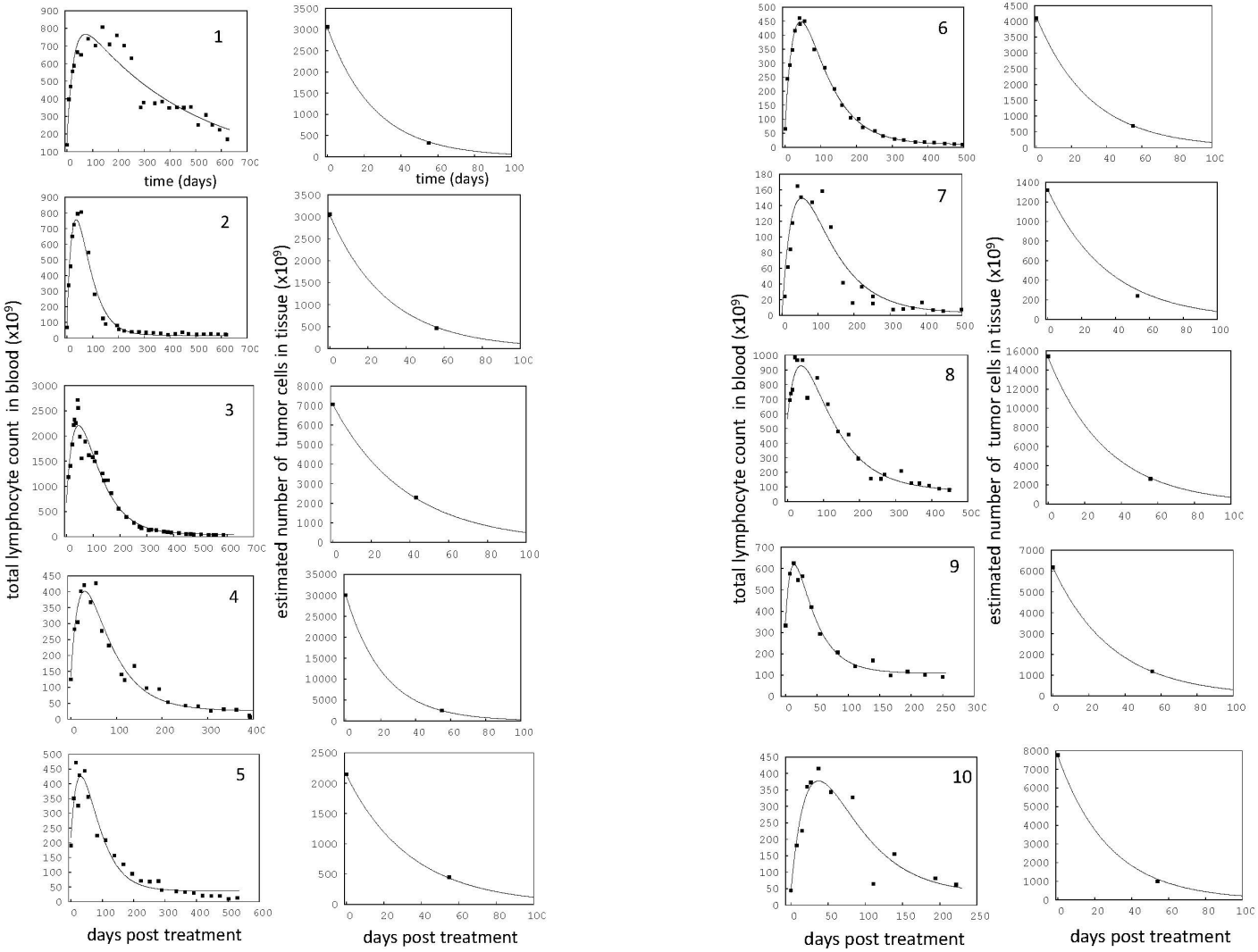
- cells in tissues

$$\frac{dx}{dt} = -mx - d_1(x - c)$$

- cells in blood => absolute lymphocyte counts

$$\frac{dy}{dt} = mx - d_2 y$$

Fitting


1
2
3
4
5
6
7
8
9
10
time (days)
days post treatment
total lymphocyte count in blood (x10⁹)
estimated number of tumor cells in tissue (x10⁹)

Parameter Estimates

patient	d_2 (d^{-1})	d_1 (d^{-1})	m (d^{-1})	α (d^{-1})	x_0 ($x10^9$)	y_0 ($x10^9$)	% redistr.
1	0.002	0.027	0.0096	0.037	3034	153	25.9
2	0.022	0.015	0.0177	0.033	3064	58	50
3	0.014	0.012	0.0146	0.026	7044	674	52.6
4	0.016	0.047	0.0009	0.047	30209	120	1.9
5	0.018	0.022	0.0095	0.032	2143	217	29.4
6	0.014	0.027	0.0061	0.033	4083	73	18.2
7	0.010	0.022	0.0056	0.028	1294	3	19.6
8	0.011	0.032	0.0023	0.034	15452	521	6.9
9	0.047	0.033	0.0088	0.042	6156	358	19.3
10	0.018	0.035	0.0034	0.039	7711	38	8.8
average	**0.017**	**0.027**	**0.008**	**0.035**	**8019**	**221**	**23.3**
st. dev.	**0.011**	**0.010**	**0.005**	**0.006**	**8799**	**226**	**17.0**

d_2 = death rate of CLL cells in blood;

d_1 = death rate of CLL cells in tissue;

m = rate of redistribution of tissue cells to blood;

α = overall nodal decline rate, i.e. rate at which cells disappear from the tissue due to redistribution + death, i.e. $\alpha=m+d_1$;.

x_0 = total body number of CLL cells in tissue;

y_0 = total body number of CLL cells in blood;

% redistr = % of pre-treatment tissue tumor burden that is redistributed.

Death rates

In tissue: $d_1 = 0.027 \pm 0.01\ days^{-1}$

2.7% ± 0.99% of the cells die per day in tissue

In blood: $d_2 = 0.017 \pm 0.012\ days^{-1}$

1.7% ± 1.1% of the cells die per day in the blood

Important message: Higher death rate in tissue than in blood

Compare to death rate in absence of treatment

In tissue: $d_1 = 0.027 \pm 0.01\ days^{-1}$

2.7% ± 0.99% of the cells die per day in tissue

treatment increases death rate 5-fold

In blood: $d_2 = 0.017 \pm 0.012\ days^{-1}$

1.7% ± 1.1% of the cells die per day in the blood

treatment increases death rate 3-fold

Previous estimate in the absence of treatment:

0.5% of cells died per day

Death rates vs redistribution rate

In tissue: $d_1 = 0.027 \pm 0.01\ days^{-1}$

2.7% ± 0.99% of the cells die per day in tissue

In blood: $d_2 = 0.017 \pm 0.012\ days^{-1}$

1.7% ± 1.1% of the cells die per day in the blood

Redistribution rate: m = 0.008 ± 0.005 $days^{-1}$

Important message: Redistribution rate significantly smaller than death rates
i.e. redistribution not main effect of drug

Death rates vs redistribution rate

In tissue: $d_1 = 0.027 \pm 0.01\ days^{-1}$

2.7% ± 0.99% of the cells die per day in tissue

In blood: $d_2 = 0.017 \pm 0.012\ days^{-1}$

1.7% ± 1.1% of the cells die per day in the blood

Redistribution rate: m = 0.008 ± 0.005 $days^{-1}$

The percentage of the tissue CLL cell population that was re-distributed into the blood was **23.3 ± 17%. - relatively small fraction**

Conclusion #1

- Ibrutinib kills cells in tissues at significant rate
- Not just death by neglect

Heterogeneity in treatment responses => correlation with genetic risk factors?

del 13q: good response with chemo-immunotherapy

Trisomy 12: intermediate response with chemo-immunotherapy

del 11q: intermediate response with chemo-immunotherapy

del 17p: ineffective response with chemo-immunotherapy

Heterogeneity in treatment responses => correlation with genetic risk factors?

del 13q: good response with chemo-immunotherapy

Trisomy 12: intermediate response with chemo-immunotherapy

del 11q: intermediate response with chemo-immunotherapy

del 17p: ineffective response with chemo-immunotherapy

Mutated CLL: good response

Unmutated CLL: bad response

Heterogeneity in treatment responses => correlation with genetic risk factors?

- Patient cohort discussed so far received previous chemo-immuno therapy
- Repeat analysis with a treatment-naïve patient cohort
- Compare **unmutated CLL** (U-CLL, more virulent type) and **mutated CLL** (M-CLL, less virulent type).

JCI insight

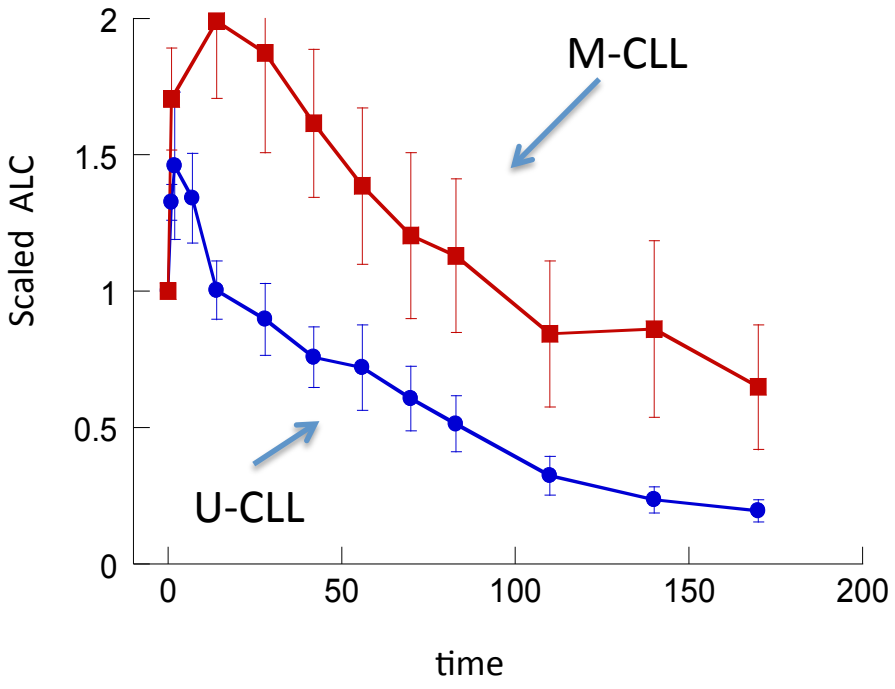
CLINICAL MEDICINE

Leukemia cell proliferation and death in chronic lymphocytic leukemia patients on therapy with the BTK inhibitor ibrutinib

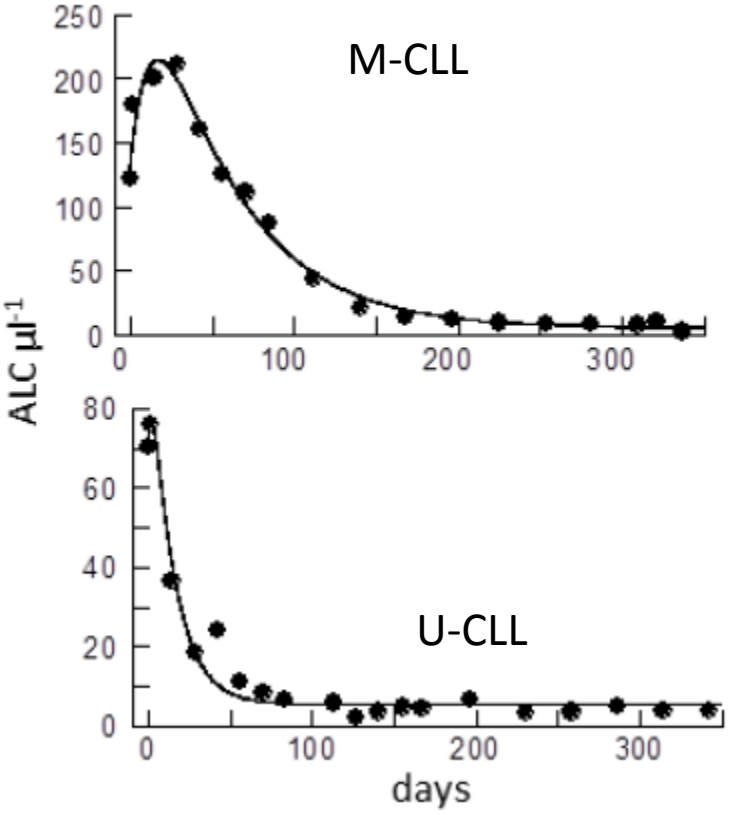
Jan A. Burger,[1] Kelvin W. Li,[2] Michael J. Keating,[1] Mariela Sivina,[1] Ahmed M. Amer,[3] Naveen Garg,[3] Alessandra Ferrajoli,[1] Xuelin Huang,[4] Hagop Kantarjian,[1] William G. Wierda,[1] Susan O'Brien,[1] Marc K. Hellerstein,[5] Scott M. Turner,[2] Claire L. Emson,[2] Shih-Shih Chen,[6] Xiao-Jie Yan,[6] Dominik Wodarz,[7] and Nicholas Chiorazzi[6]

blood lymphocyte dynamics during therapy in U-CLL and M-CLL

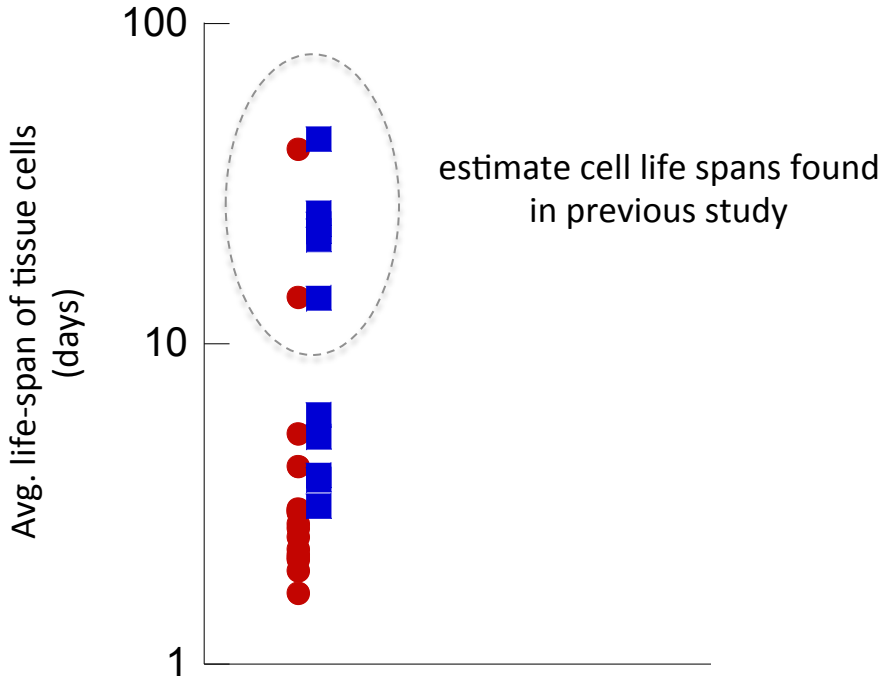
average dynamics of whole cohort



individual example and model fits



Estimated tissue death rates



M-CLL

average tissue cell death rate:
12.06±9.42%

U-CLL

average tissue cell death rate:
28.88±11.33%

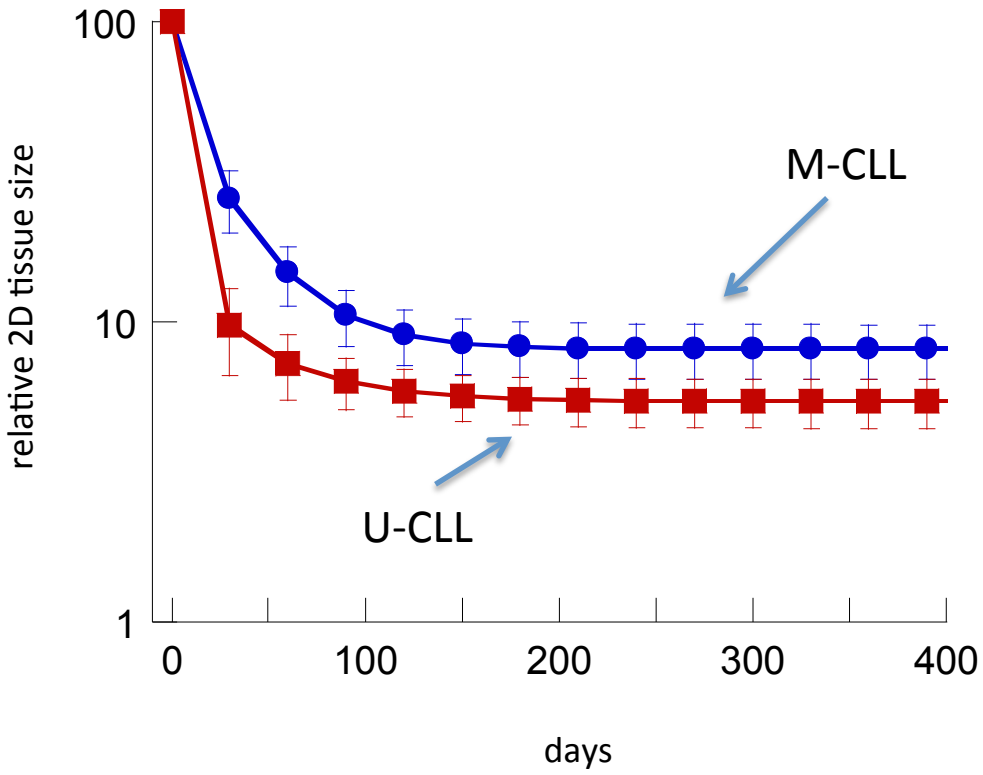
statistically significant difference, p=0.0017

Some patients have tissue cell death rates consistent with previous cohort (10-50 days life-span)

In other patients tissue cells die much faster (1-10 days life span)

Significantly faster tissue cell death rates are observed in patients with higher risk factors (U-CLL)

Simulated average tissue dynamics in U-CLL and M-CLL



Conclusion #2

More virulent U-CLL responds faster than less virulent M-CLL

Need to re-evaluate meaning of traditional risk factors in context of new targeted treatments

Another important outcome of these studies:

For individual patients, we can measure kinetic parameters that characterize the response to ibrutinib.

- total tumor size at the start of treatment
- tissue and blood cell death rates during treatment
- redistribution rate of cells from tissue to blood

Patient-specific parameters can be plugged into mathematical models to make individualized predictions about treatment outcome

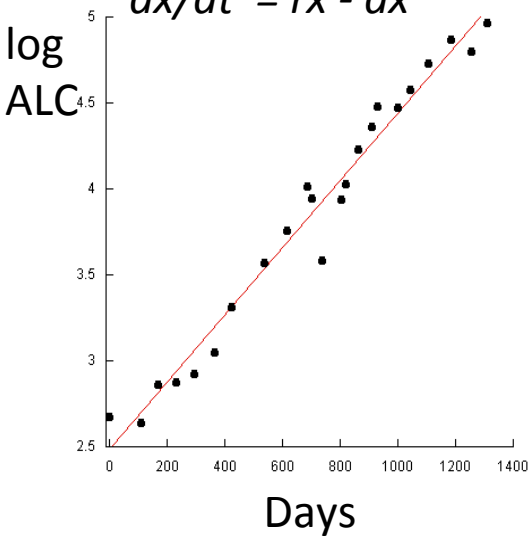
=> Towards using evolutionary theory for personalized medicine

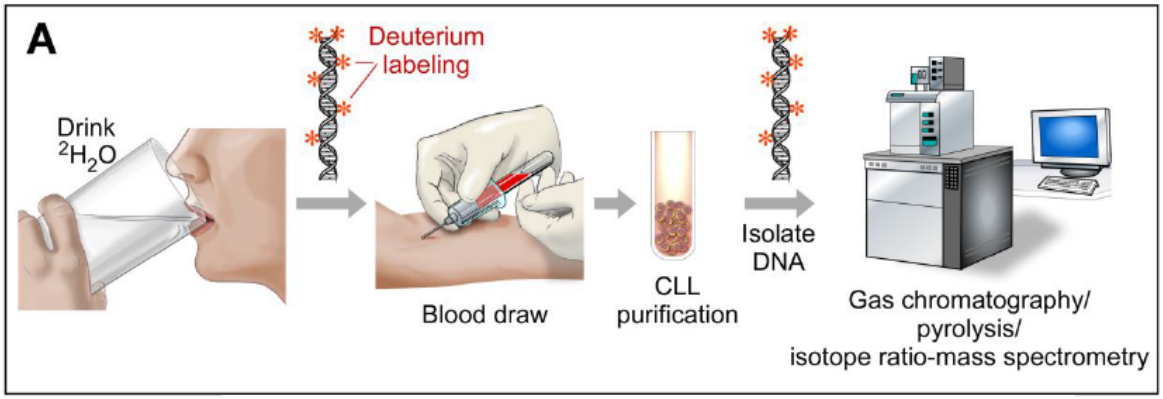
=> Explore this in the context of resistance evolution

We can also measure patient-specific parameters before treatment

exponential growth:

$dx/dt = rx - dx$





This study used deuterium, a nonradioactive isotope detectable by mass spectrometry, that was administered in the form of deuterated "heavy" water (^2H_2O), to label newly synthesized DNA of dividing cells in vivo (26). The kinetic profiles identi-

- dynamics of label uptake and dilution allows you to estimate the division rate of cells, *r*.
- knowing the overall growth rate and the division rate of cells allows us to estimate the death rate of cells from exponential growth rate, *d*.

Evolutionary Dynamics of Resistance against ibrutinib

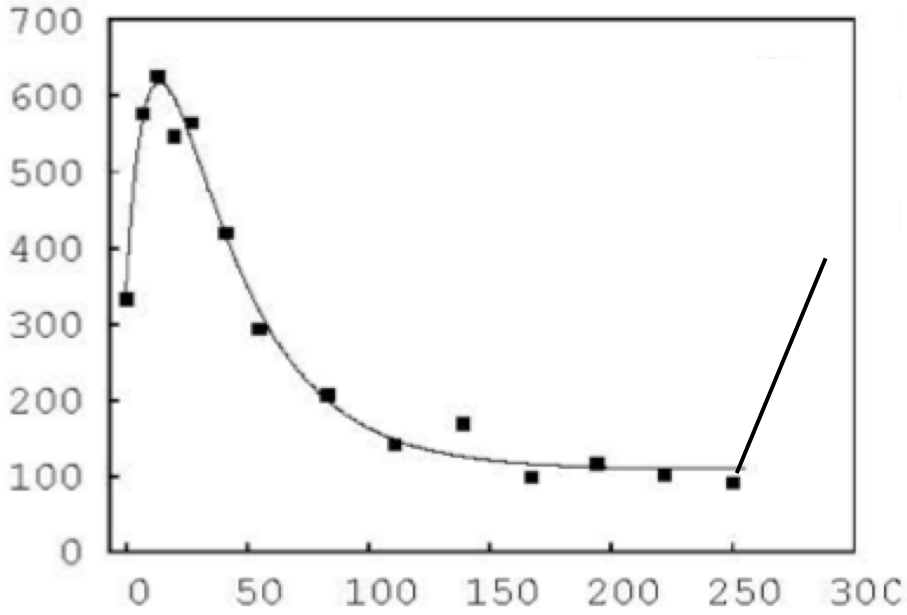
PNAS

Evolution of ibrutinib resistance in chronic lymphocytic leukemia (CLL)

Natalia L. Komarova[a,b,1], Jan A. Burger[c], and Dominik Wodarz[a,b]

[a]Department of Mathematics and [b]Department of Ecology and Evolutionary Biology, University of California, Irvine, CA 92697; and [c]Department of Leukemia, MD Anderson Cancer Center, Houston, TX 77230

Edited* by Francisco J. Ayala, University of California, Irvine, CA, and approve



Resistance mechanisms

Furman et al 2014
Woyach et al 2014
Sharma et al 2016

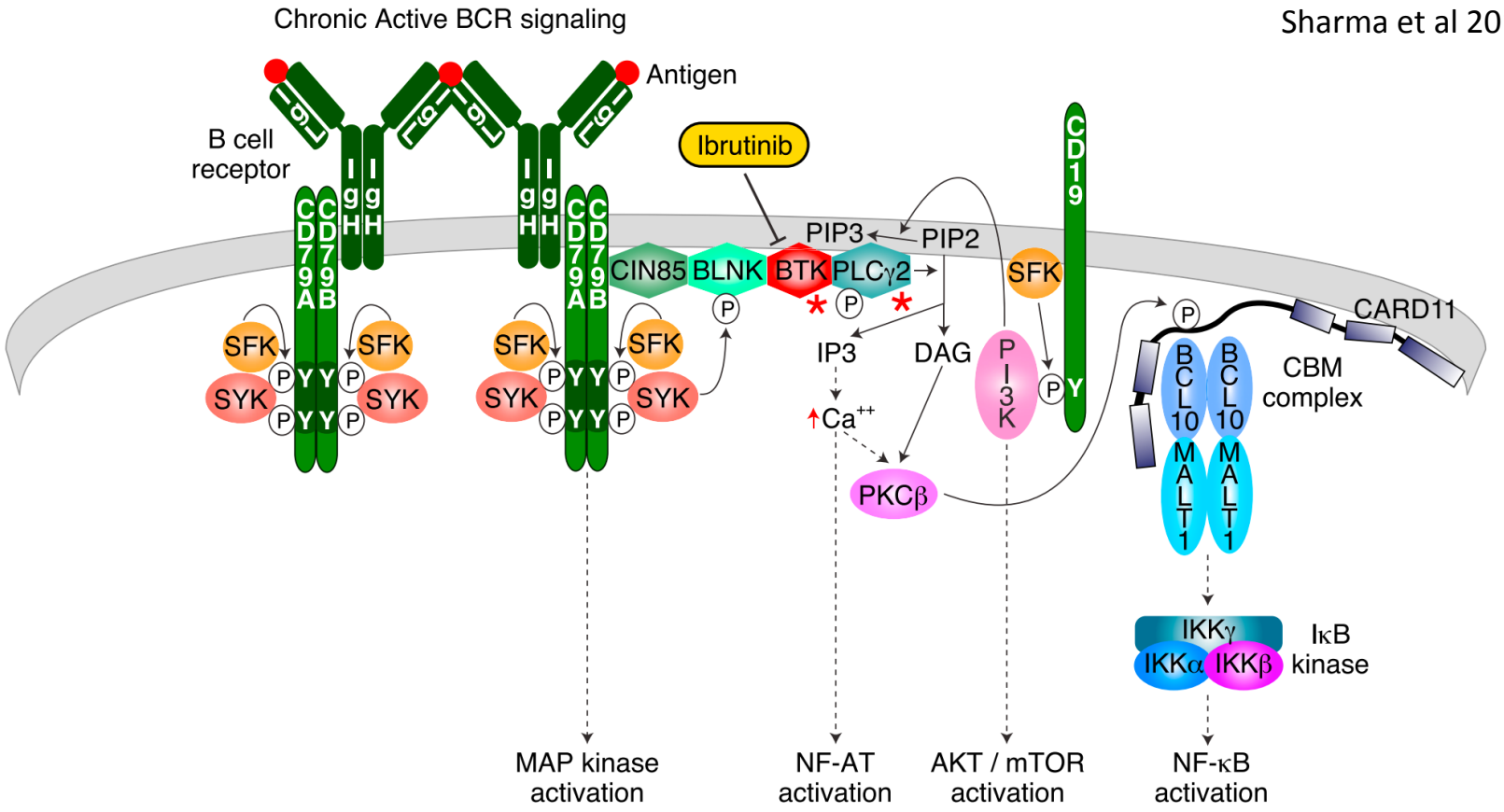


Figure 1. B Cell Receptor Signaling in Malignant B Cells
Chronic active BCR signaling is shown. Ibrutinib is shown to inhibit BTK. Red asterisks denote signaling effectors that are the target of ibrutinib resistance mutations in CLL patients.

Resistance mechanisms

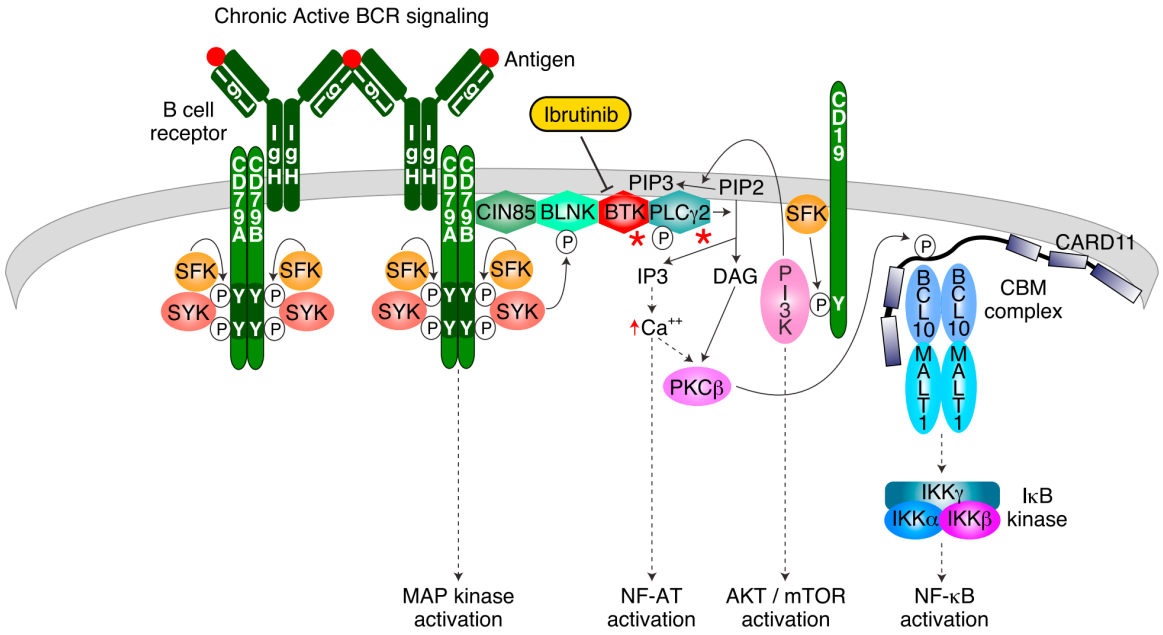


Figure 1. B Cell Receptor Signaling in Malignant B Cells
Chronic active BCR signaling is shown. Ibrutinib is shown to inhibit BTK. Red asterisks denote signaling effectors that are the target of ibrutinib resistance mutations in CLL patients.

Furman et al 2014
Woyach et al 2014
Sharma et al 2016

Resistant mutants likely neutral, perhaps slightly advantageous

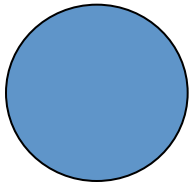
Construct and parameterize evolutionary mathematical model and ask the following questions:

1. Can we predict time of resistance-induced disease relapse?

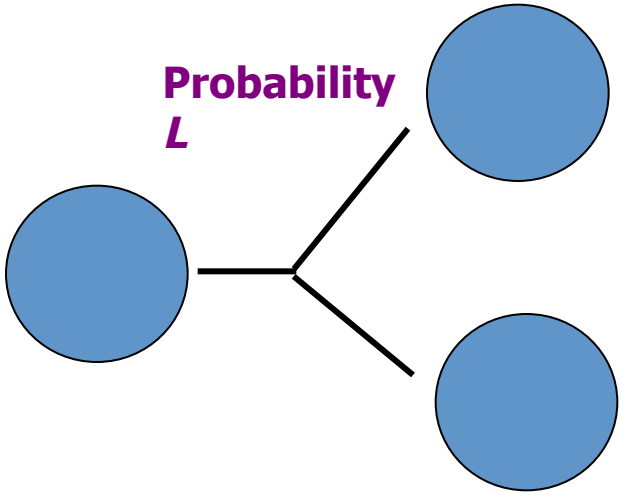
2. If predicted relapse time is short, can we suggest approaches to prolong it?

Mathematical model – stochastic birth death process

cancer cell



Mathematical model



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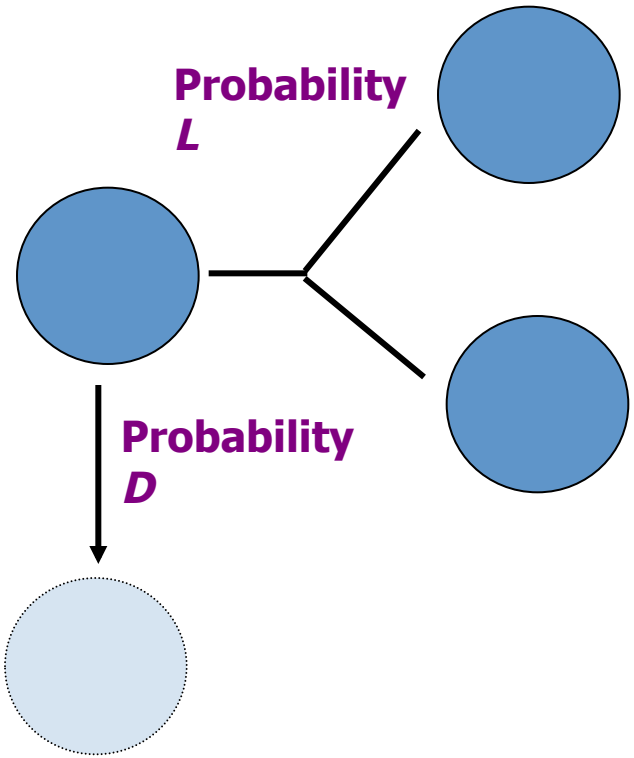

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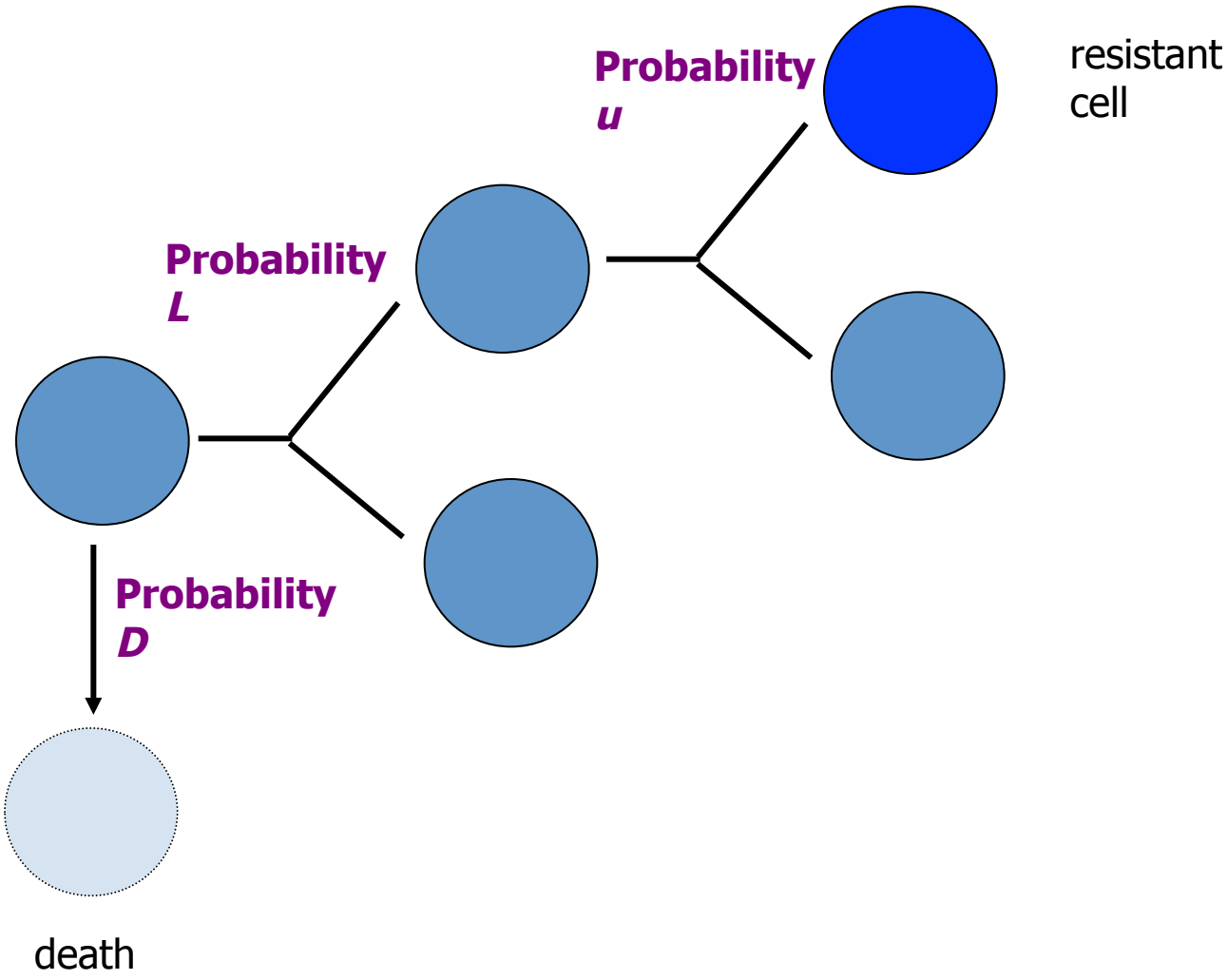
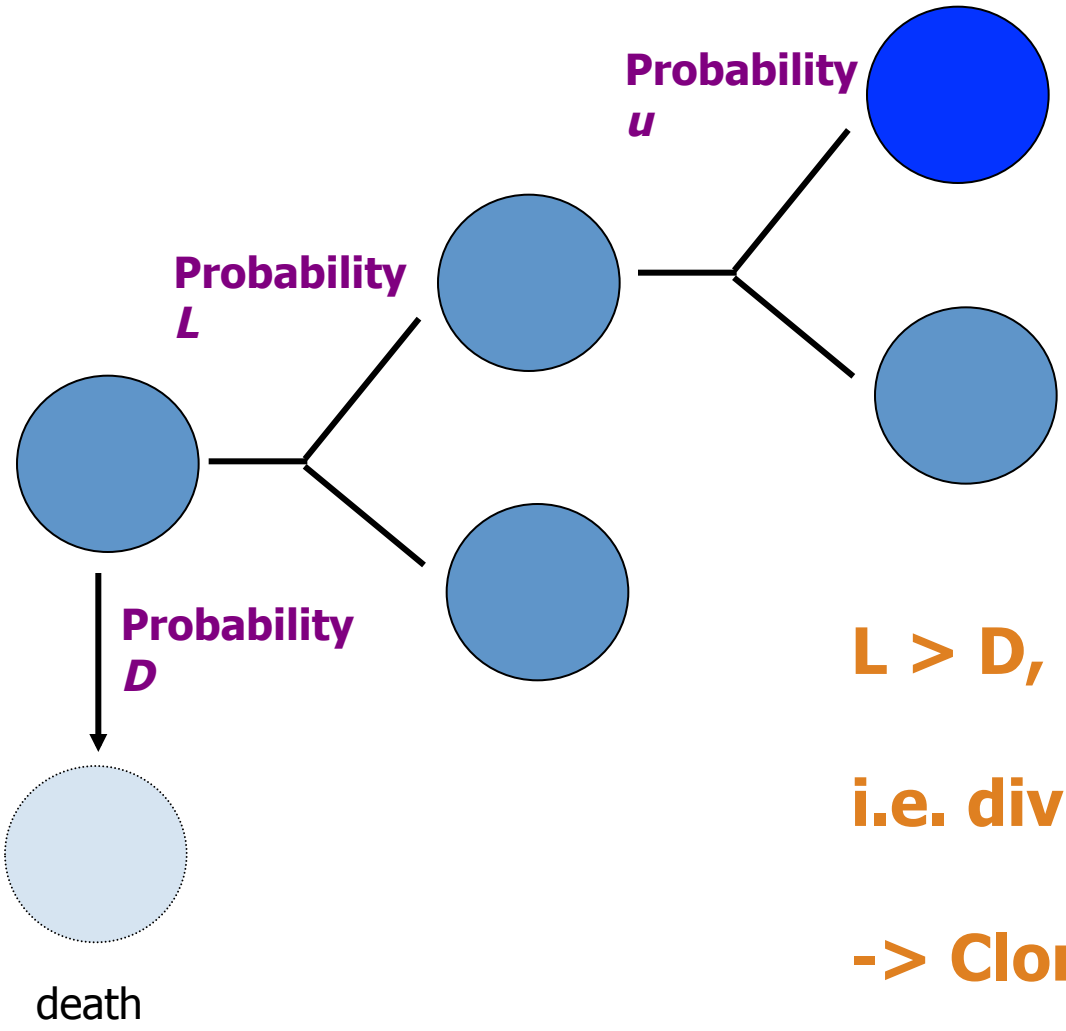


Mathematical model – growth phase

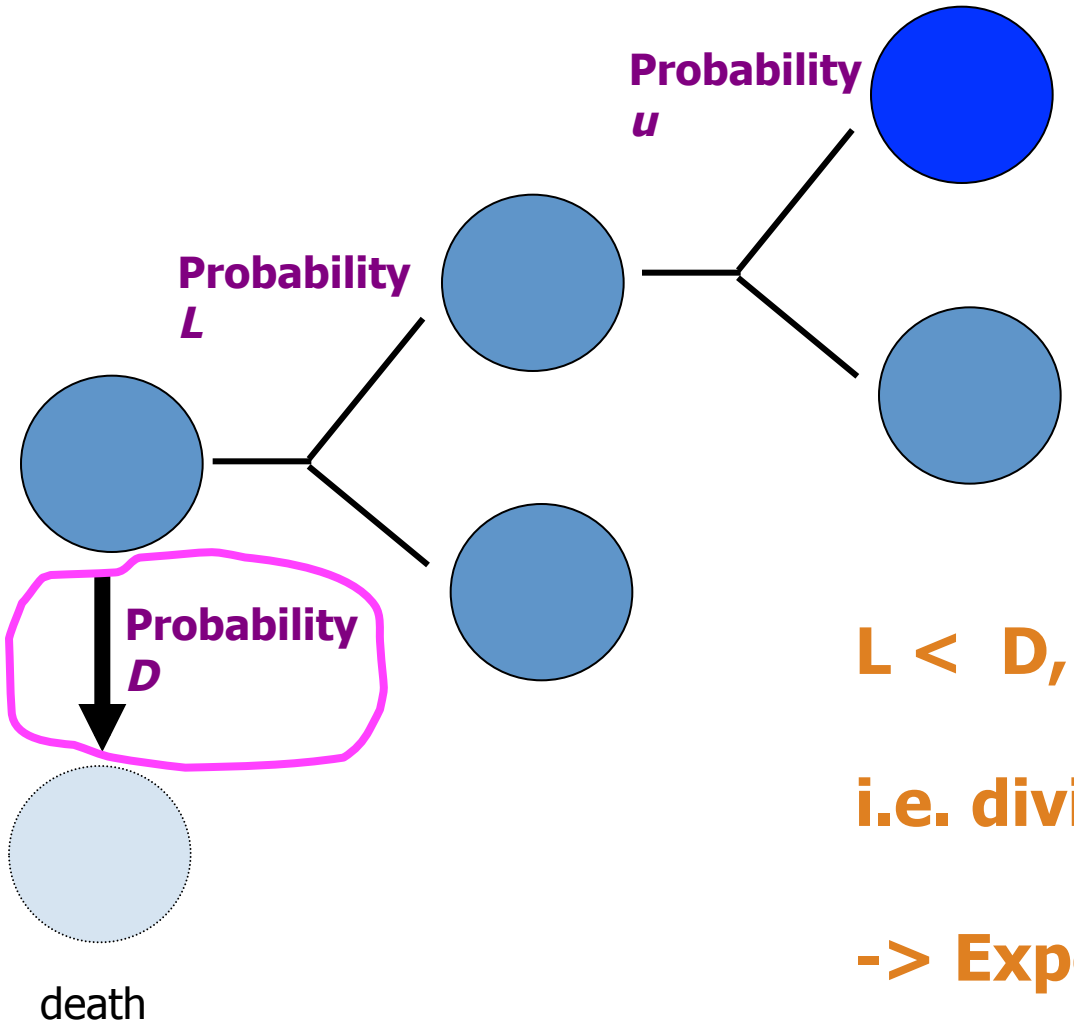


L > D,

i.e. division rate > death rate

-> Clonal Expansion

Mathematical model – treatment phase



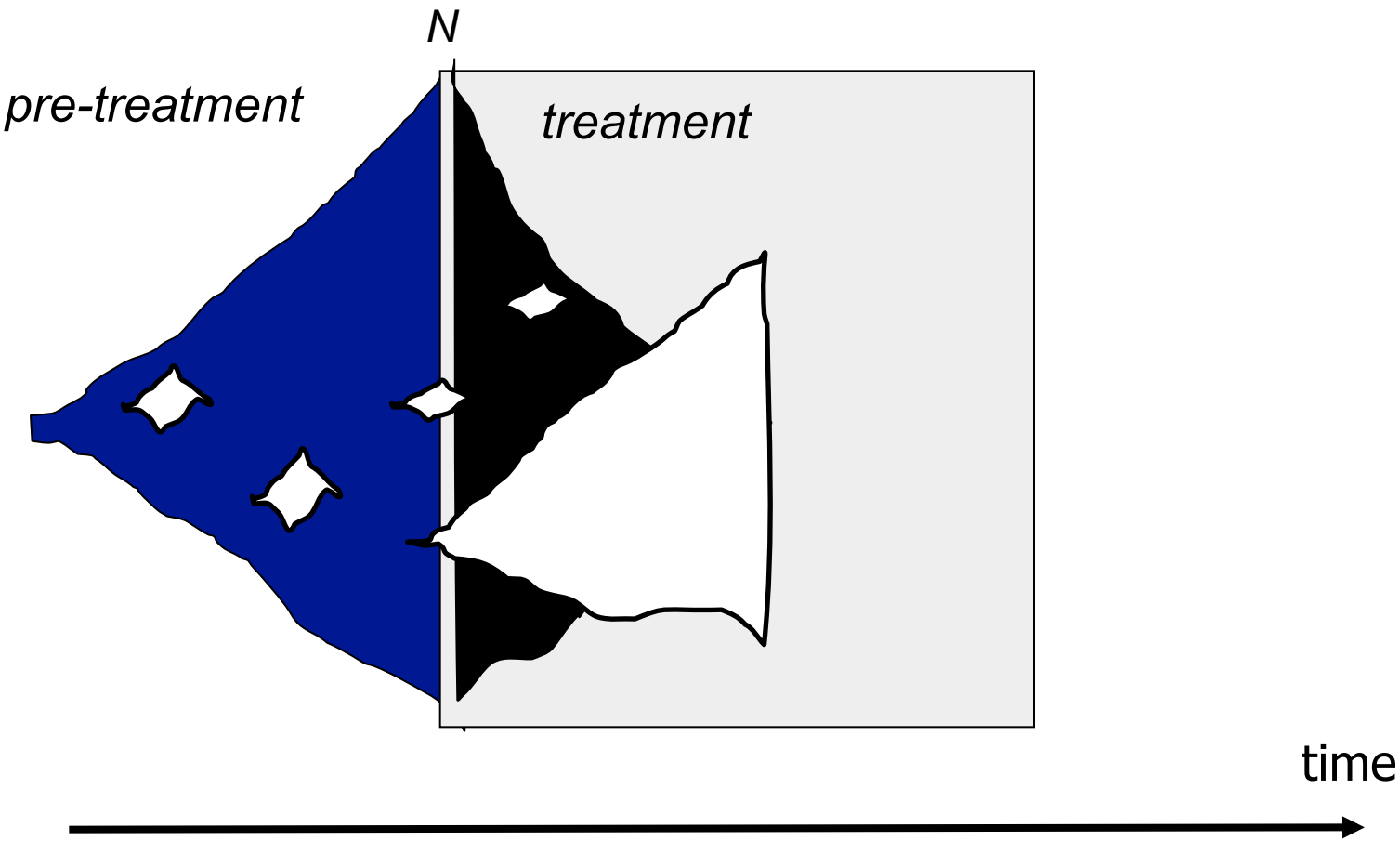
L < D,

i.e. division rate < death rate

-> Exponential Decline

Principles of model

(ii) with resistance



Parameters values for individual patients

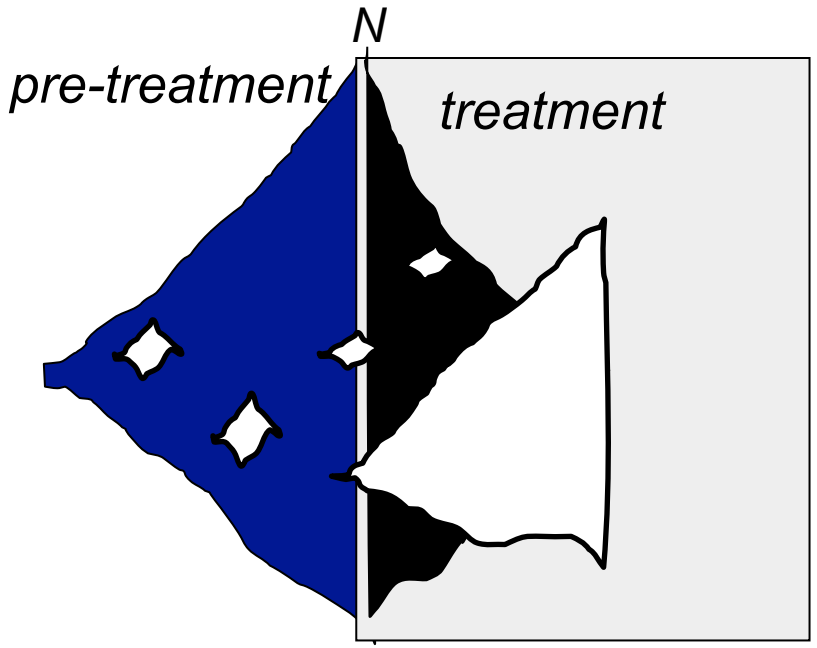
Problem: we have a limited number of patients in our cohort

Solution: Virtual patients

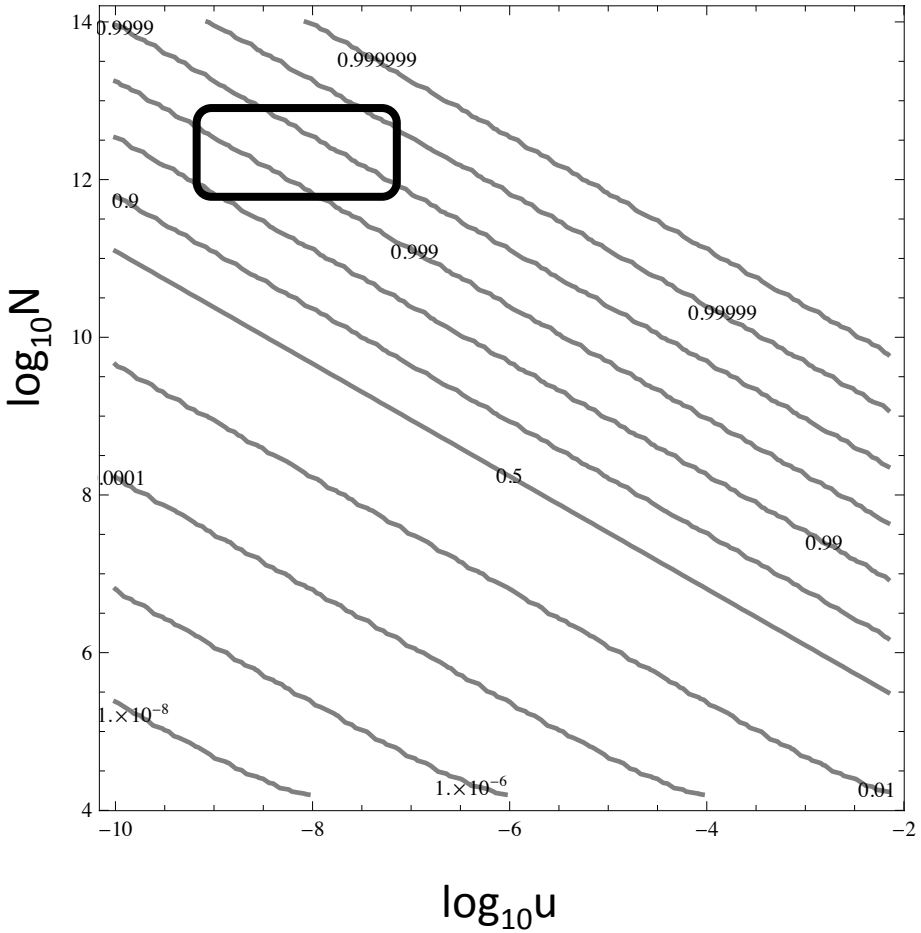
A population of 1000 artificial “patients” is simulated with parameters randomly drawn from the clinically available bounds

First Important Question

What are the chances that resistant mutants are already present at the time when treatment Is started?



Answer: Resistant mutants are almost certainly present before the start of therapy



Probability of having a mutant in a colony at detection

Number of CLL cells in tissue is 10^{12}-10^{13}

Mutation rate is 10^{-9}-10^{-8}

Drug resistant cells are almost certain to exist before detection

Clinical confirmation of theory

ARTICLE

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Clonal evolution in patients with chronic lymphocytic leukaemia developing resistance to BTK inhibition

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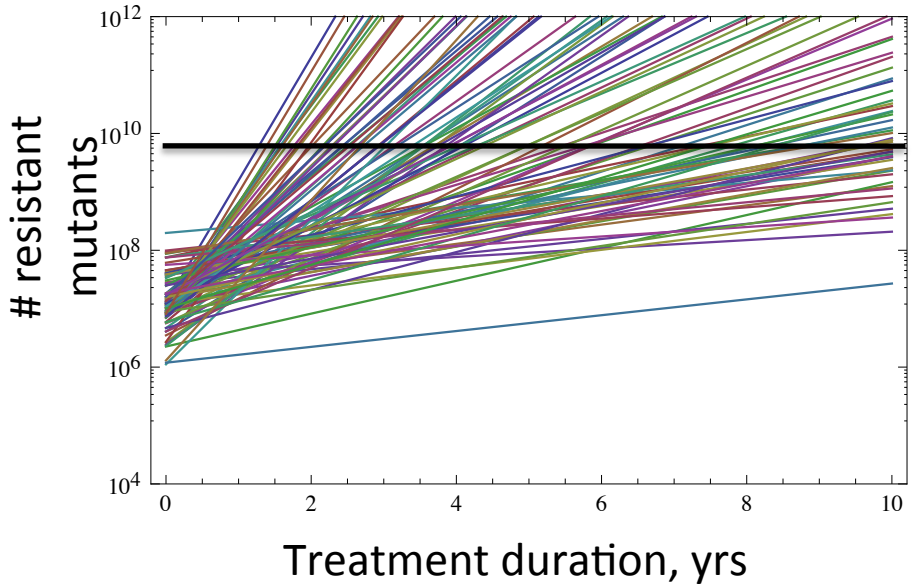
Resistance to the Bruton's tyrosine kinase (BTK) inhibitor ibrutinib has been attributed solely to mutations in *BTK* and related pathway molecules. Using whole-exome and deep-targeted sequencing, we dissect evolution of ibrutinib resistance in serial samples from five chronic lymphocytic leukaemia patients. In two patients, we detect *BTK-C481S* mutation or multiple *PLCG2* mutations. The other three patients exhibit an expansion of clones harbouring *del*(8p) with additional driver mutations (*EP300, MLL2* and *EIF2A*), with one patient developing *trans*-differentiation into CD19-negative histiocytic sarcoma. Using droplet-microfluidic technology and growth kinetic analyses, we demonstrate the presence of ibrutinib-resistant subclones and estimate subclone size before treatment initiation. Haploinsufficiency of TRAIL-R, a consequence of *del*(8p), results in TRAIL insensitivity, which may contribute to ibrutinib resistance. These findings demonstrate that the ibrutinib therapy favours selection and expansion of rare subclones already present before ibrutinib treatment, and provide insight into the heterogeneity of genetic changes associated with ibrutinib resistance.

Next Question

Given that mutants pre-exist at start of therapy, can we predict how long it takes for them to grow sufficiently to cause relapse?

- Predict the average number of mutants at treatment start.
- Predict how long it takes them to reach detectable levels.

Growth dynamics of resistant mutants: Heterogeneity of (virtual) patient populations



Each line shows the **average** growth trajectory **for a given parameter combination** (i.e. for an individual virtual patient)

- Although resistance is predicted to be present with certainty, its dynamics are very different for different patients

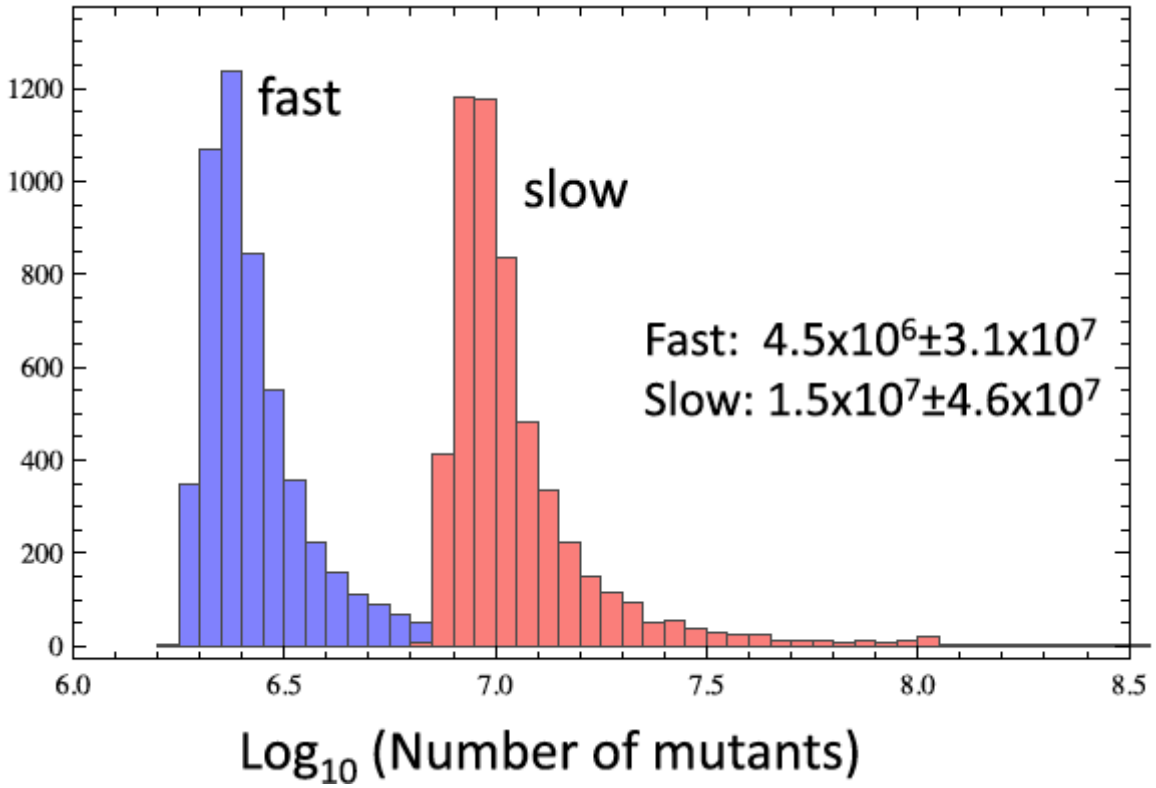
- The only variables are CLL growth rates and population size at detection

Do **average** growth trajectories provide meaningful information?

- For a particular patient (a particular parameter combination), growth of CLL cells is a stochastic process.
- How wide are the **variations** within one parameter set?
- Can we use mean numbers as guidance?

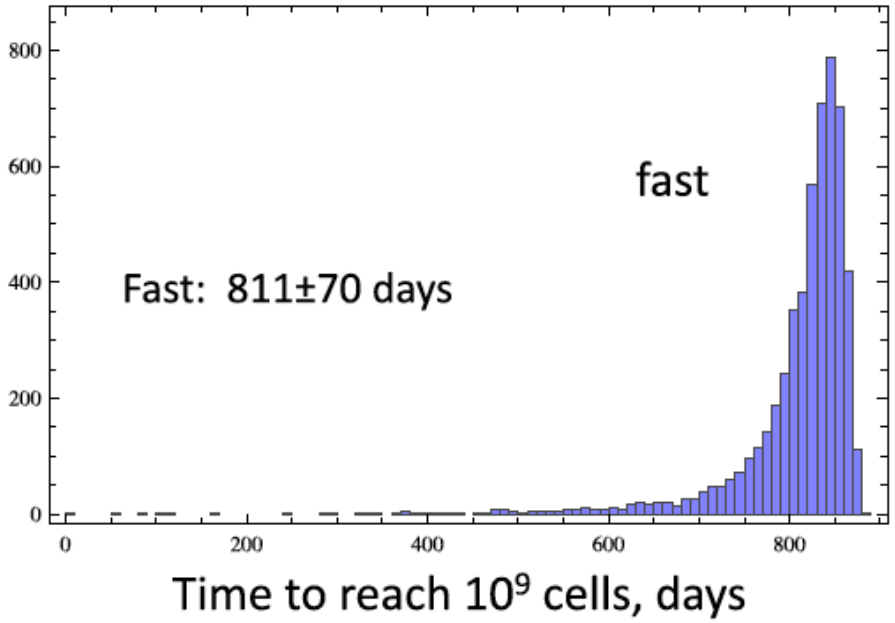
Do mean numbers provide meaningful information?

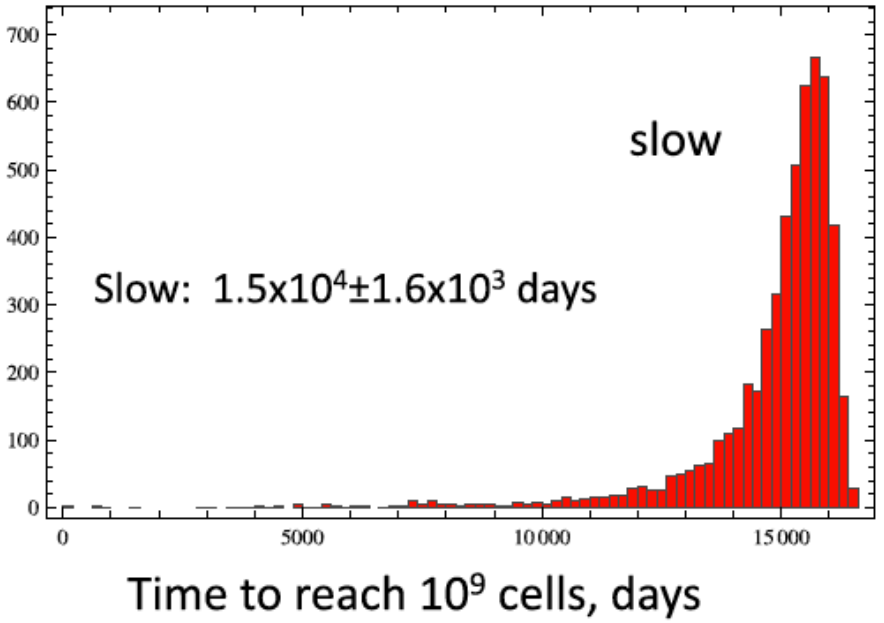
(a) Number of mutants present at start of treatment: large variation



Do mean numbers provide meaningful information?

(b) Time until resistance is detected: small variation





this is the clinically important quantity

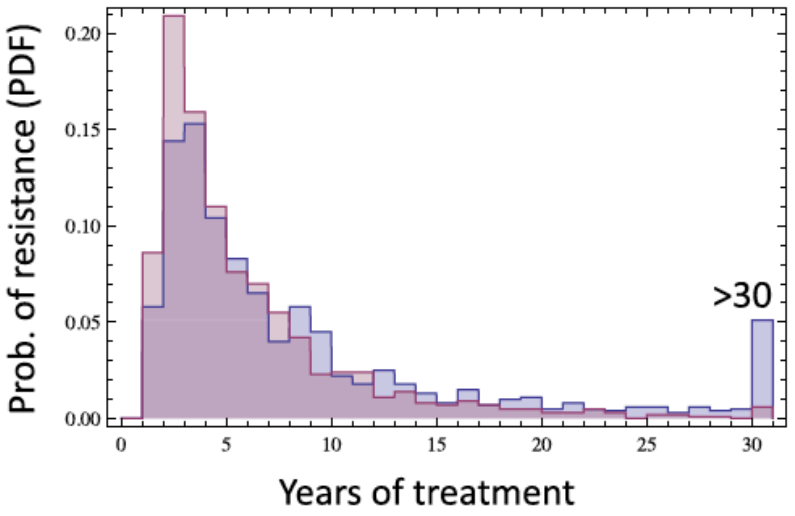
Do mean numbers provide meaningful information?

For a particular patient, if the parameters are known, we can reliably predict the time until disease relapse (from a mathematical standpoint).

Predictions about time of disease relapse

Standard Ibrutinib therapy

Timing	% patients
Resistance before 2 years	6%
Resistance before 5 years	46%
Resistance before 10 years	75%
No resistance after 30 years	5%



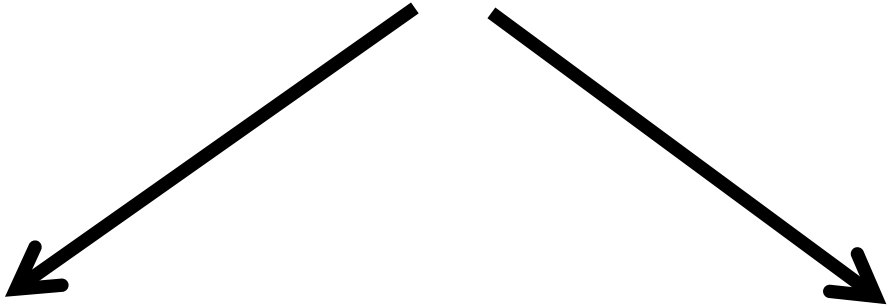
Mean time to resistance generation is 9 years if resistant mutants are neutral

or 5 years if they are slightly advantageous

Personalized prediction

measure kinetic parameters in individual patient

predict how long ibrutinib monotherapy can maintain control



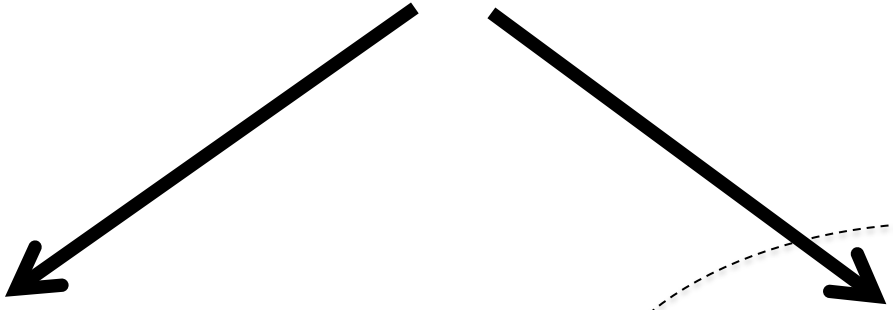
Long time, e.g. > 10 years
=> therapy ok

Short time, e.g. 1 year
=> inbrutinib monotherapy is insufficient
=> other approaches needed.

Personalized prediction

measure kinetic parameters in individual patient

predict how long ibrutinib monotherapy can maintain control



Long time, e.g. > 10 years
=> therapy ok

Short time, e.g. 1 year
=> inbrutinib monotherapy is insufficient
=> other approaches needed.

Personalized improvement of therapy

Can we use the model to identify treatment approaches to prolong ibrutinib-mediated control of the disease?

Possible strategies to overcome resistance

- Early treatment (treat upon diagnosis, not watch & wait)
- Combining 2 tyrosine kinase inhibitors (toxicity might be problematic)
- “Debulking” = first use chemo-immunotherapy, followed by ibrutinib

Possible strategies to overcome resistance

- ~~Early treatment (treat upon diagnosis, not watch & wait)~~
- ~~Combining 2 tyrosine kinase inhibitors (toxicity might be problematic)~~
- "Debulking" = first use chemo-immunotherapy, followed by ibrutinib

Can resistance be prevented by any of these approaches?

=> according to calculations, resistance cannot be prevented

Debulking before ibrutinib: while it is not likely to prevent occurrence of resistant mutants, can it delay relapse?

Predictions

Standard Ibrutinib therapy

Timing	% patients
Resistance before 2 years	6%
Resistance before 5 years	46%
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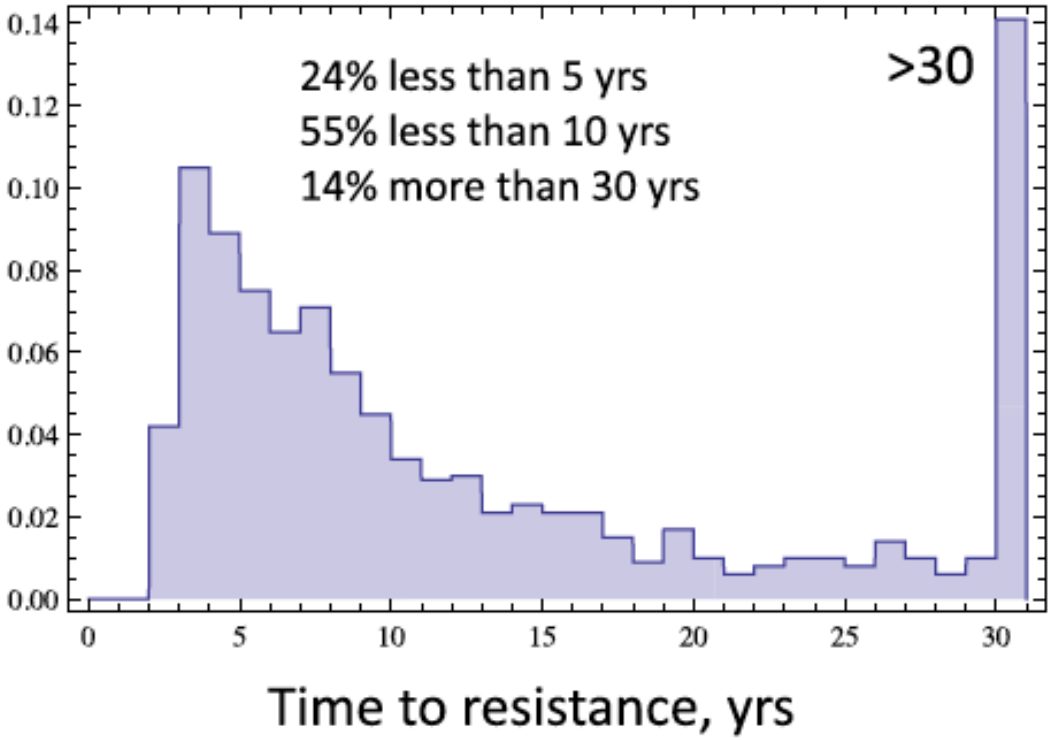
No resistance after 30 years	5%

"Debulking" by a factor of 1/100



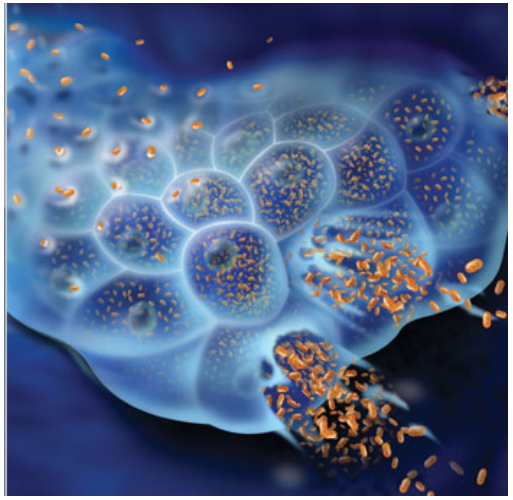
Debulking prior to ibrutinib can significantly delay the timing of relapse

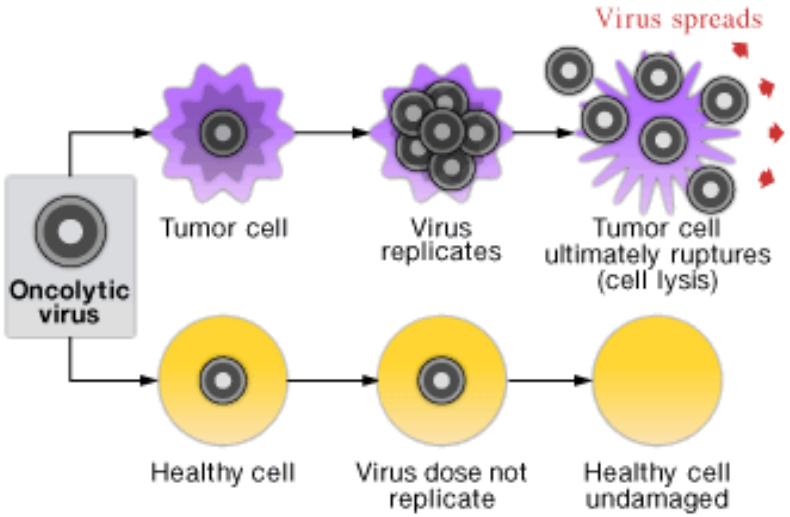
Conclusions

- Used math + clinical data to estimate patient-specific parameters
- Calculated that ibrutinib causes significant amounts of cell death in tissue, rather than just causing redistribution of tumor cells from tissue to blood
- Determined that risk factors that distinguished responsive / non-responsive patients in chemotherapy treatment might have to be re-evaluated in the context of ibrutinib
- Developed an evolutionary theory framework to make patient-specific prediction about therapy outcomes, and to compute treatment strategies to improve outcome

=> Test model predictions against clinical data
=> Explore mathematically in more detail how treatment can be improved further
=> Design clinical trial that is based on the mathematical and evolutionary foundations.

Spatial dynamics of virus spread – oncolytic viruses





Complex Dynamics of Virus Spread from Low Infection Multiplicities: Implications for the Spread of Oncolytic Viruses

Ignacio A. Rodriguez-Brenes, Andrew Hofacre, Hung Fan, Dominik Wodarz 2017

Complex Spatial Dynamics of Oncolytic Viruses In Vitro: Mathematical and Experimental Approaches

Dominik Wodarz, Andrew Hofacre, John W. Lau, Zhiying Sun, Hung Fan, Natalia L. Komarova 2012

Early infection and spread of a conditionally replicating adenovirus under conditions of plaque formation

Andrew Hofacre [a], Dominik Wodarz [b], Natalia L. Komarova [c], Hung Fan [a] 2012

Experimental system: Adenovirus *AdEGFPuci* growing on 293 cells

- Virus related to well-established oncolytic vitus ONYX-015
- Virus is labeled with green fluorescent protein so we can not only track numbers of infected cells, but also spatial patterns.
- Cells are spatially arranged such that a source cell is most likely to transmit virus to directly neighboring target cells (2D).

Andy Hofacre and Hung Fan (UCI)

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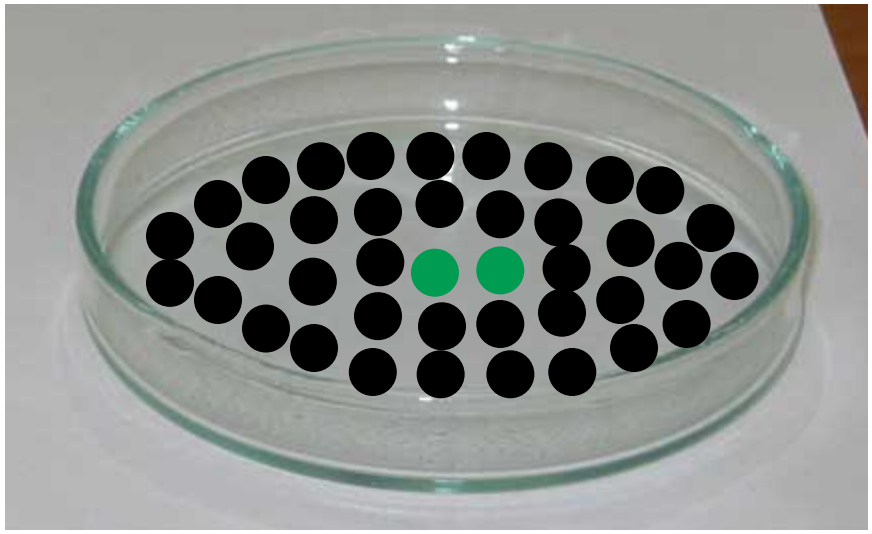

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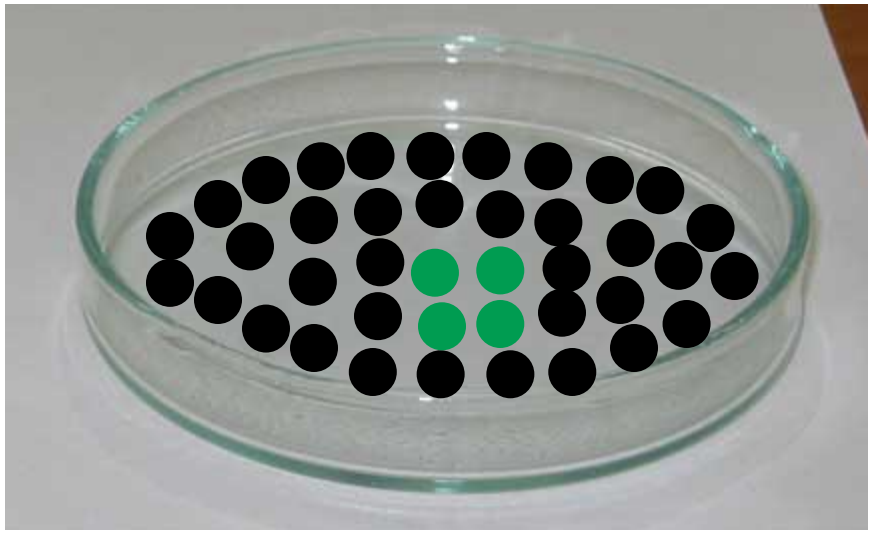
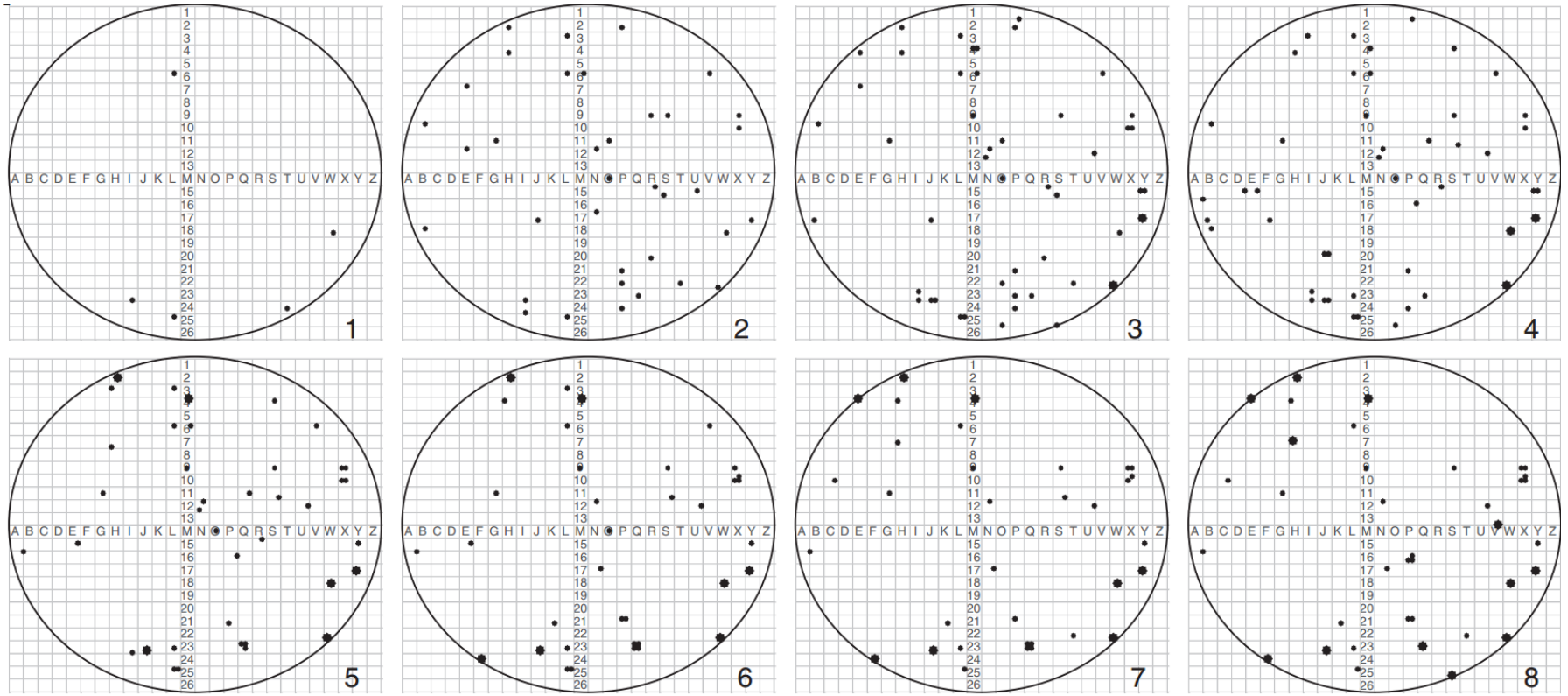


Experiments: follow individual infection foci



Hofacre et al 2012, Virology

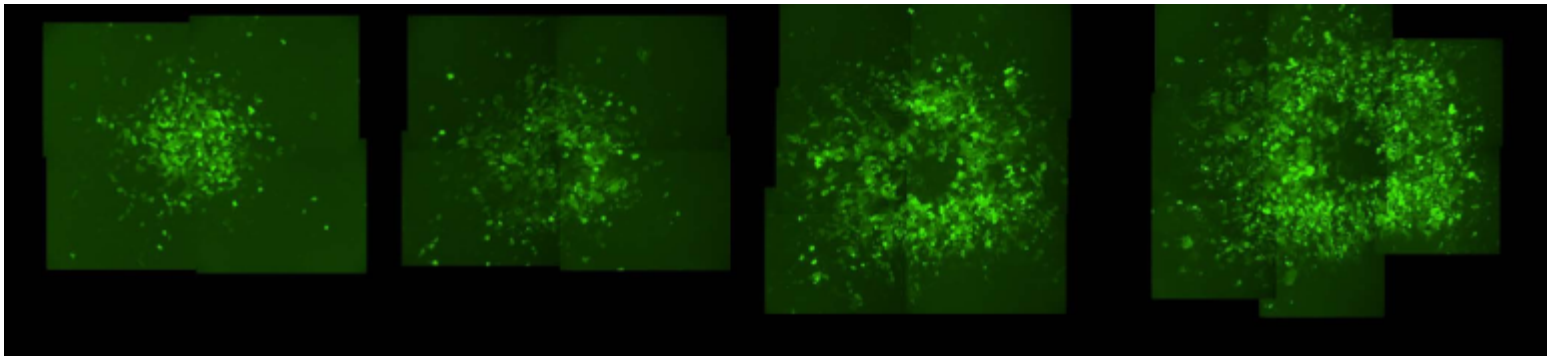
Longer-term growth patterns

2 types of growth patterns observed

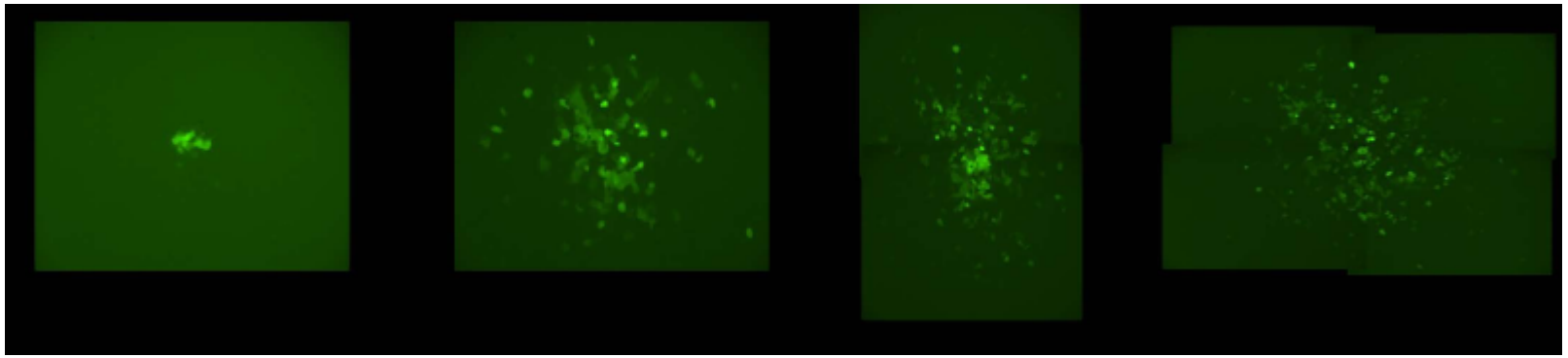
Traditional plaque or “ring” or “robust growth

“diffuse growth pattern” or “limited growth”

Robust growth / Ring structure



Limited / Diffusive growth



Wodarz et al 2012, PLoS Computational Biology

How can such growth patterns be explained?

=> We turn to a stochastic, agent-based computational model

Agent-based model

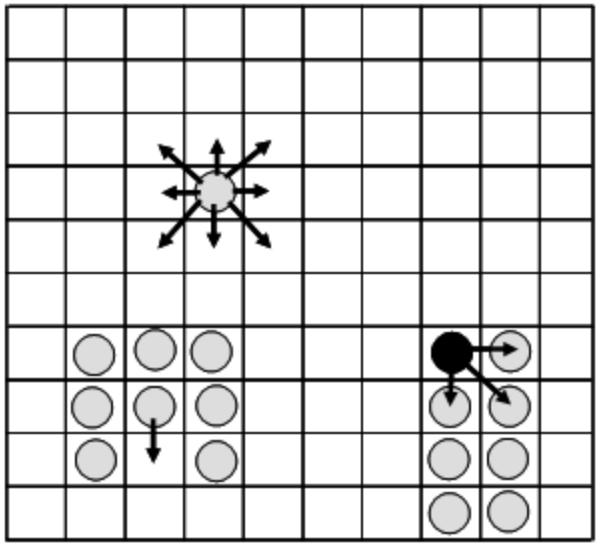
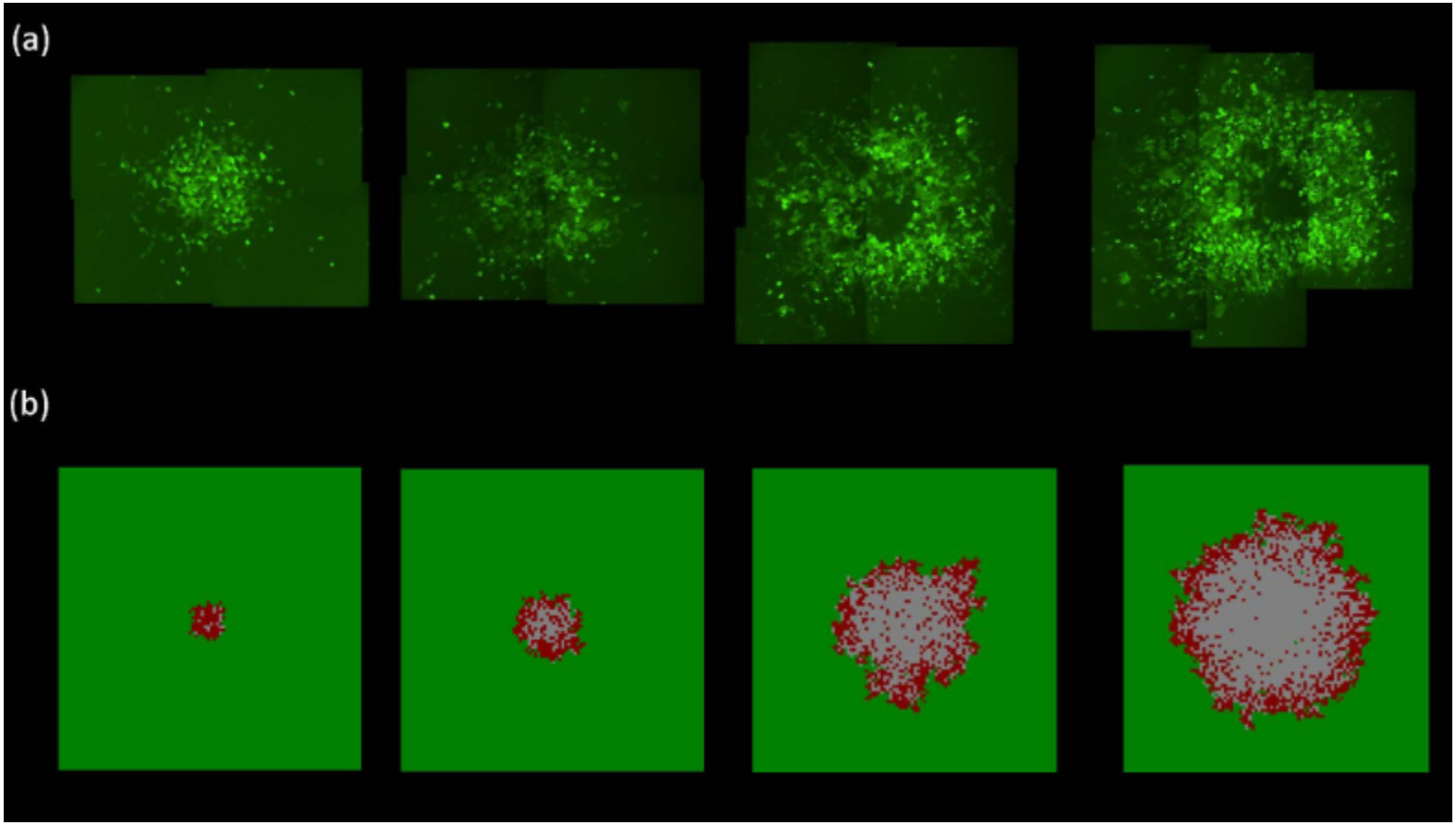


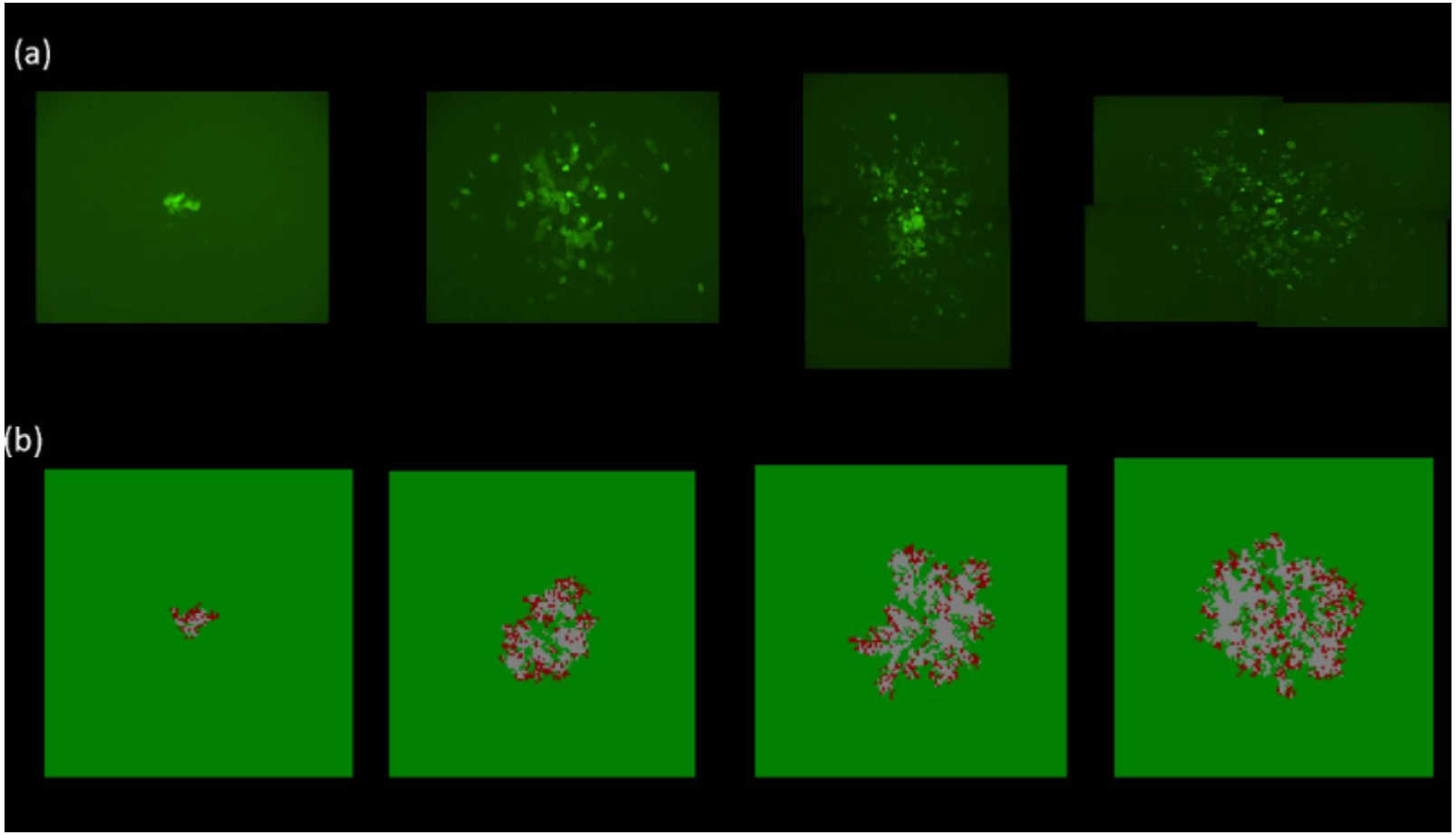
Figure 2: *Diagram explaining the cellular automaton. Gray=uninfected cell, Black=infected cell. During cell division, one of the daughters occupies one of the nearest neighboring slots. If a slot is already occupied, the a daughter cell cannot move there. If all neighboring slots are occupied, no division occurs. An infected cell can pass on the virus to uninfected cells which are located in the nearest neighboring slots. The virus cannot be passed on to a slot which is not occupied. For further details, see text.*

Model / Data



faster viral replication rate, longer life-span of infected cells

Model / Data

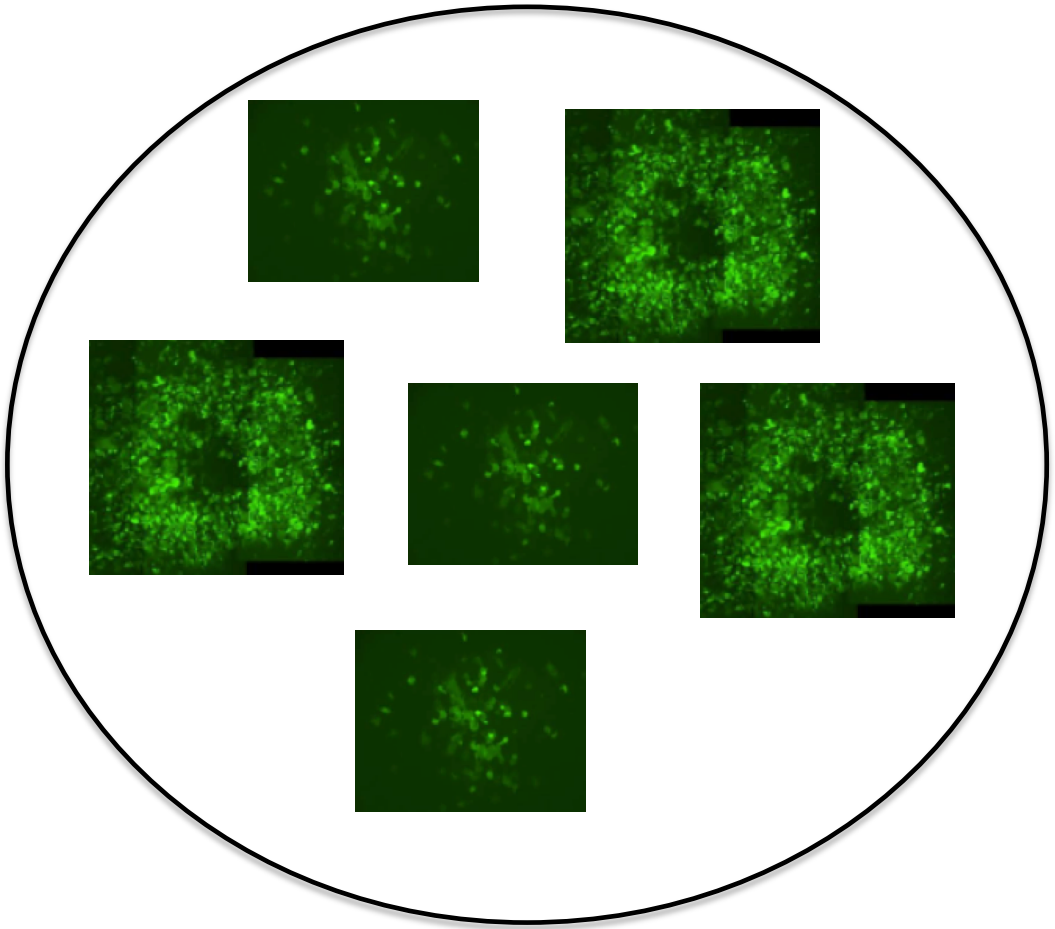


slower viral replication rate, shorter life-span of infected cells

Further complexities

According to the model, the different spatial patterns can be brought about differences in parameters

But experiments indicate, the the situation is more complex



Different patterns are observed in same culture, i.e. same virus-cell combinations, and identical experimental conditions.

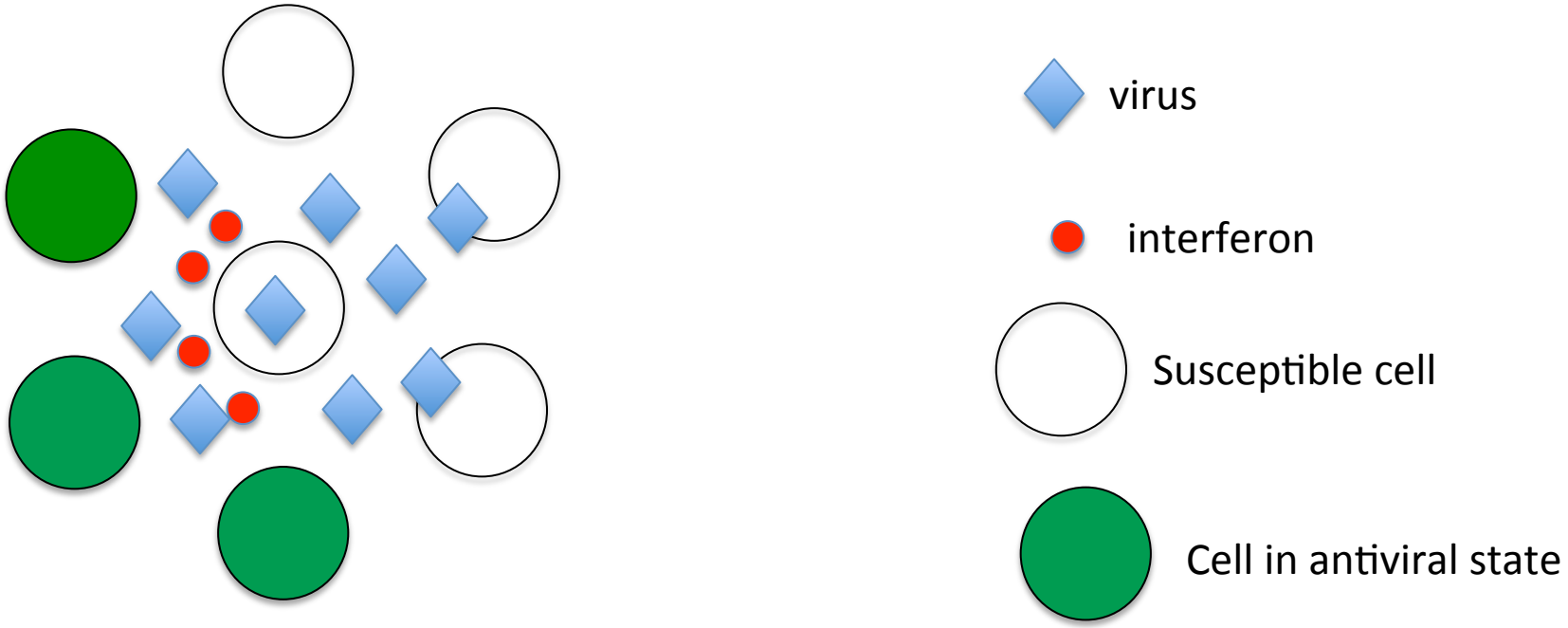
about 50% ring structure

50% disperse pattern

Why?

Exploring possible mechanisms:

1. Race between virus and antiviral factors (interferon)



With *AdEGFPuci* infection of 293 cells, data indicate that a limited anti-viral state is induced in cells.

Rodriguez-Brenes et al 2017, PLoS Computational Biology

Experimental test: Inhibiting interferon increases % robust growth

TABLE 1

EFFECT OF VALPROIC ACID ON VIRAL SPREAD[1]

	Spreading Infections		
VPA (mM)	Limited	Robust	% Robust
0	131	120	48
10	30	54	64
15	16	30	67

[1]Ad-293 cells were infected with AdEGFPuci under conditions of plaque formation, in the presence of different concentrations of valproic acid. At 14 days post-infection the numbers of spreading infections with limited and robust patterns were scored by fluorescent microscopy. The experiment was repeated at least three times with similar results.

Inhibiting interferon: increases % robust

TABLE 2

EFFECTS OF ANTI - IFNAR2 AND RAPAMYCIN ON VIRAL SPREAD[1]

Treatment	Spreading Infections		
	Limited	Robust	% Robust
None	74	68	48
	5	5	50
Anti – IFNAR2	26	105	80
	5	17	77
Rapamycin	93	379	80
	15	48	76

[1]Ad-293 cells were infected with AdEGFPuci under conditions of plaque formation, in the presence of anti-IFNAR2 mAb (1 μg/ml) or rapamycin (5 ng/ml). The infections were carried out with two different concentrations of AdEGFPuci differing by ten-fold. At 14 days post-infection the numbers of spreading infections with limited and robust patterns were scored by fluorescent microscopy. Results for the lower virus inocula are shown below the results for the higher inocula. The experiment was repeated a second time with similar results.

Inhibition of interferon increases the percentage of robust infections.

Hypothesis:

- Initial race between virus spread and the spread of antiviral state explains occurrence of limited and robust spread in same dish

- Stochastic effects determine whether the antiviral response wins (limited spread) or whether the virus wins (robust spread)

Inhibition of interferon increases the percentage of robust infections.

Hypothesis:

- Initial race between virus spread and the spread of antiviral state explains occurrence of limited and robust spread in same dish

- Stochastic effects determine whether the antiviral response wins (limited spread) or whether the virus wins (robust spread)

=> Test this hypothesis with mathematical models

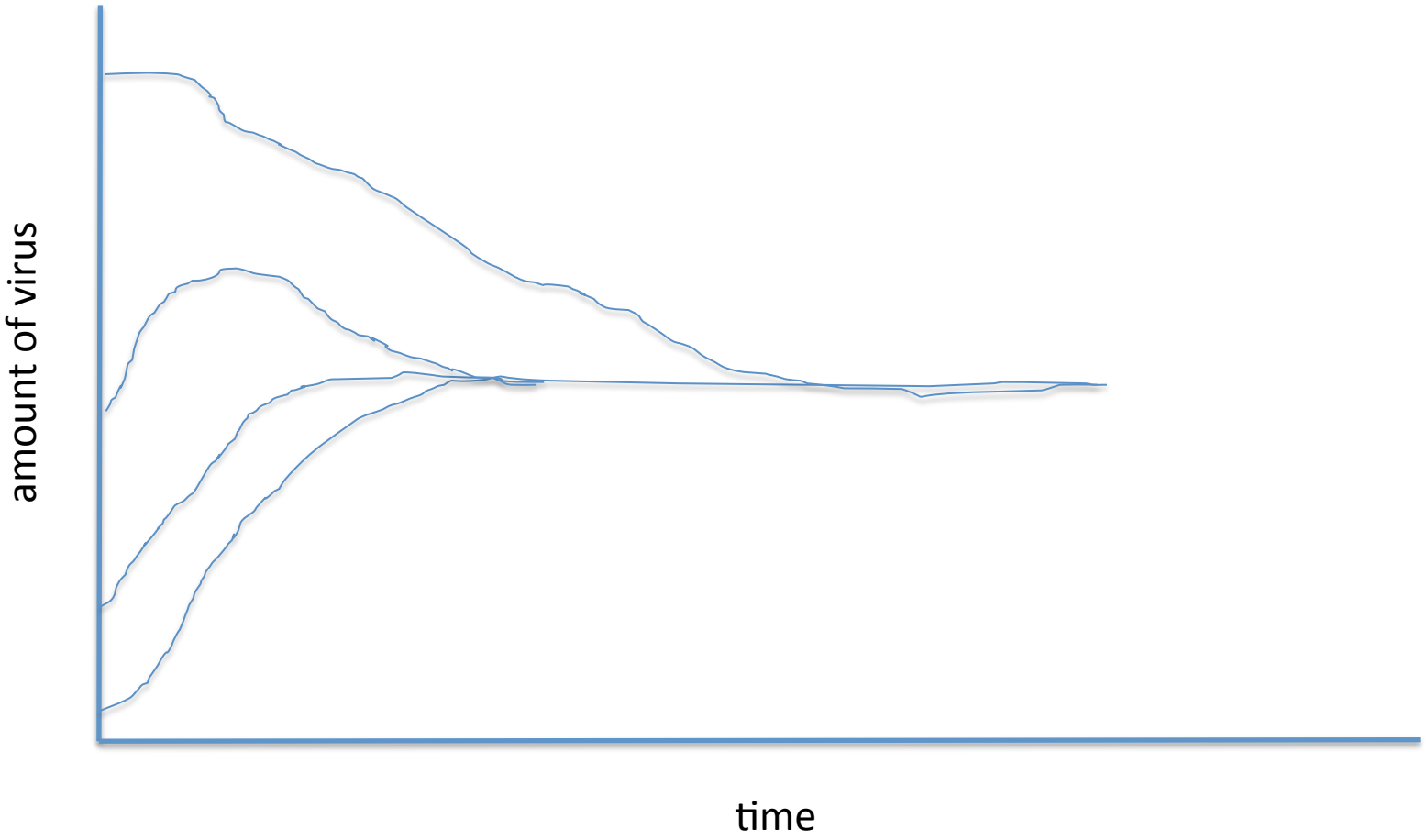
Start: Non-spatial model for analytical tractability (ODEs)

Move to: Spatial "metapopulation models, based on the ODEs

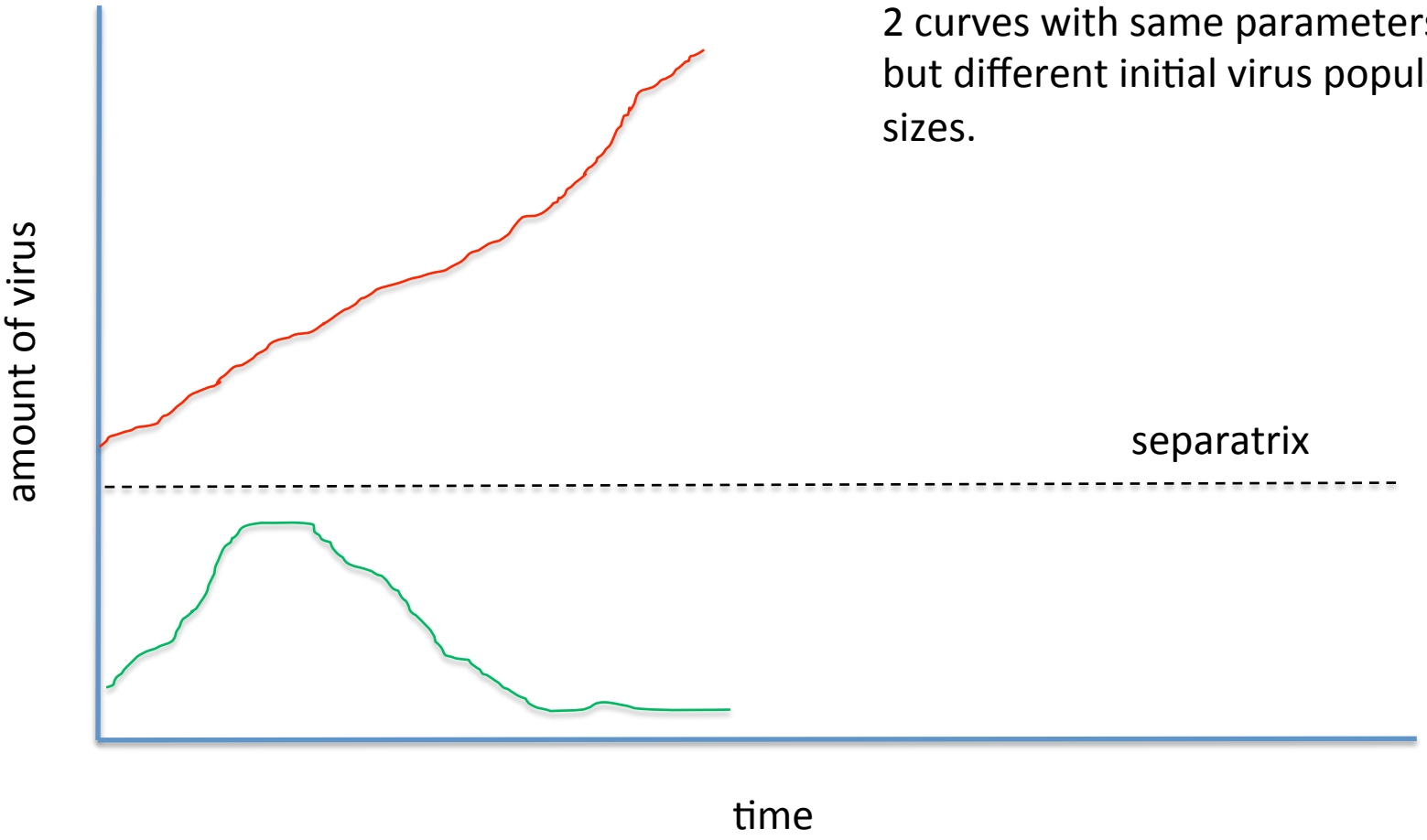
Finally: Spatial, agent-based model that tracks individual cells

What model properties are needed to see different outcomes under identical conditions?

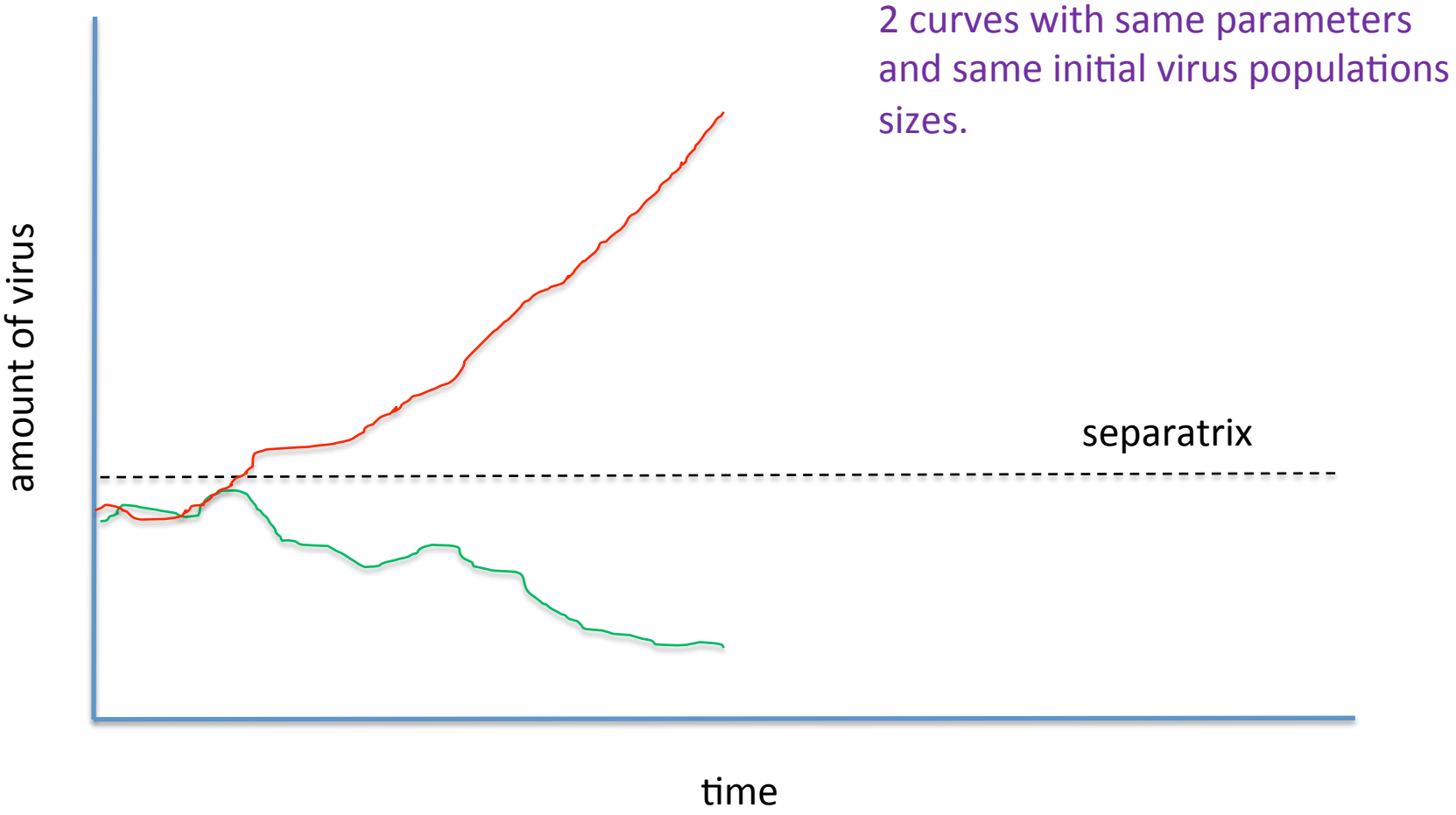
I many models, for a given set of parameters, the system always converges to the same outcome, no matter what the initial population sizes are:



Need bistability and dependence on initial conditions in model to explain data



Effect of stochasticity



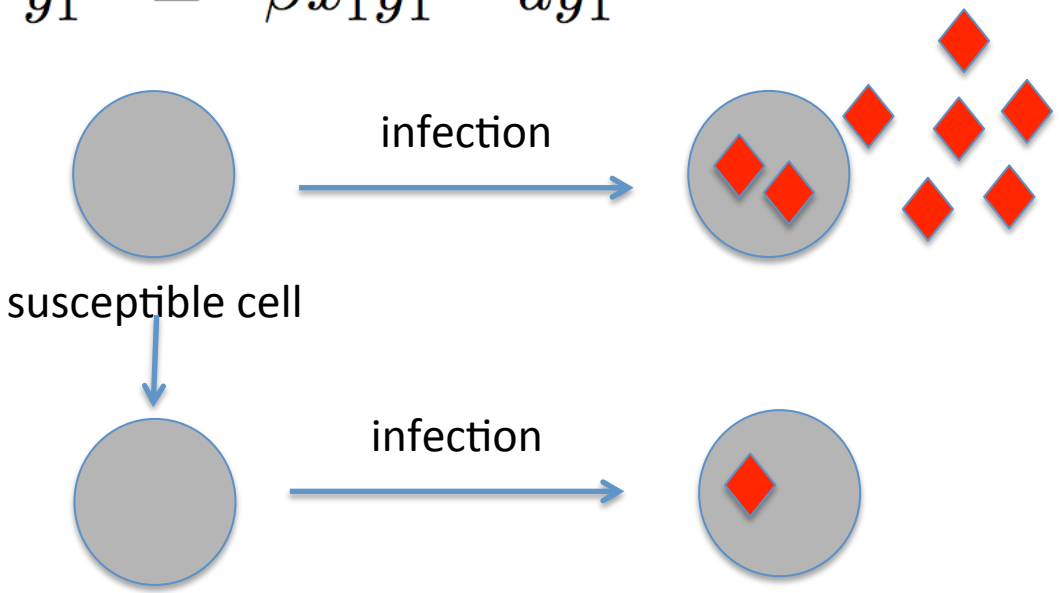
An ODE model with cells in an antiviral state

x1: susceptible cells

y1: infected cells

x0: cells in antiviral state

$$\begin{cases} \dot{x}_1 &= rx_1\left(1-\frac{x_1+x_0+y_1}{K}\right) - dx_1 + gx_0 - \beta x_1 y_1 - \gamma x_1 y_1 \\ \dot{x}_0 &= \gamma x_1 y_1 - gx_0 - dx_0 \\ \dot{y}_1 &= \beta x_1 y_1 - ay_1 \end{cases}$$



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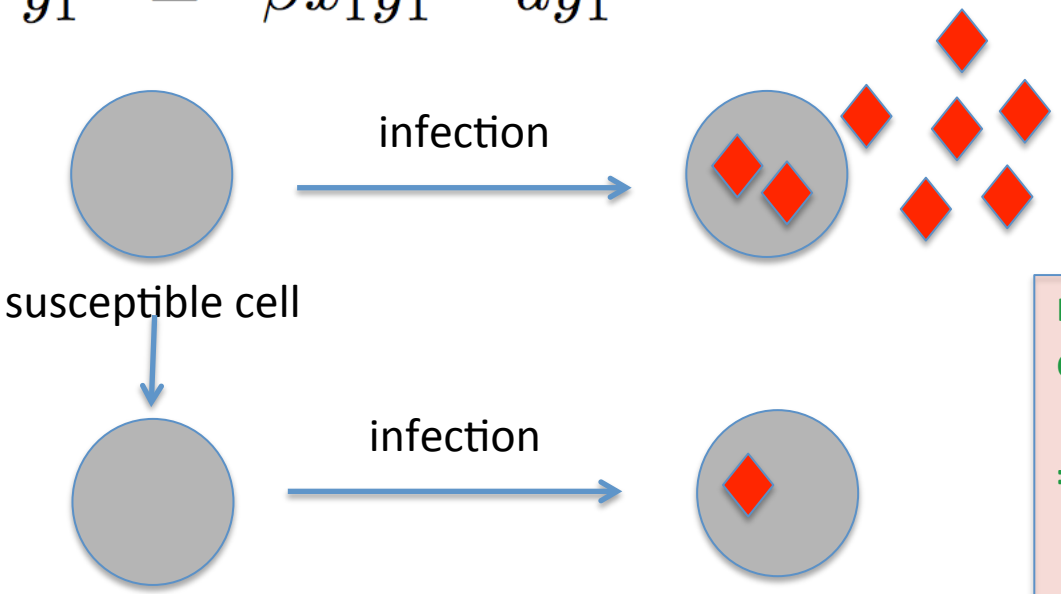


no particularly interesting dynamics observed. No bistability.

=> race between spread of virus and anti-viral state alone cannot explain our data!!!

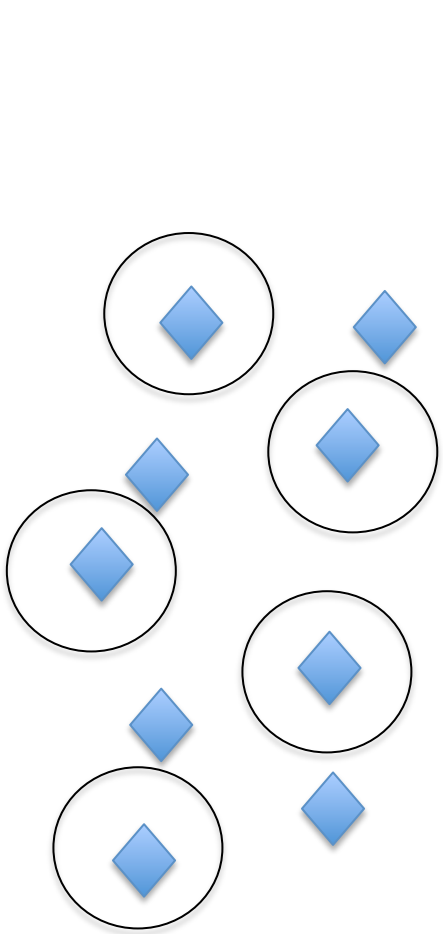
We can reject this hypothesis

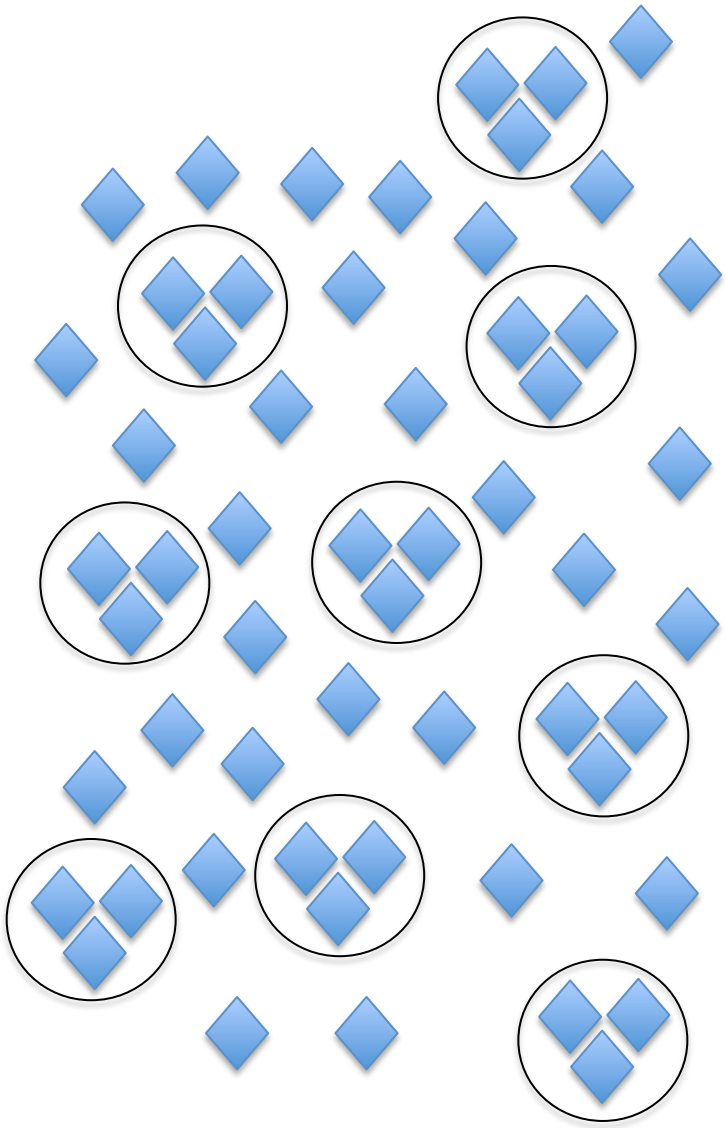
Despite experimental indications, race between virus spread and the spread of an IFN-induced antiviral state cannot explain the data.

Need additional components

So let's add some more complexity

- Multiple infection and increased replication kinetics



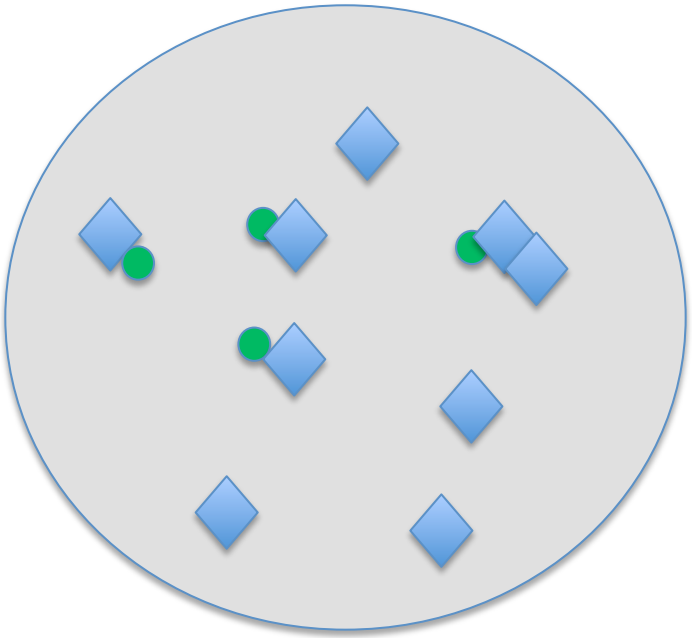


Additional assumption: Increased replication kinetics in multiply infected cells can saturate the antiviral state

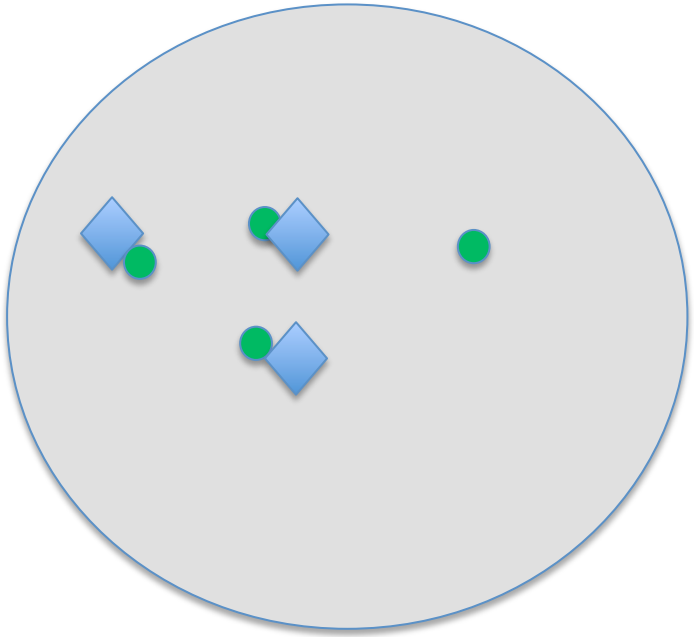
virus

Antiviral factors

Multiple infection / fast replication kinetics

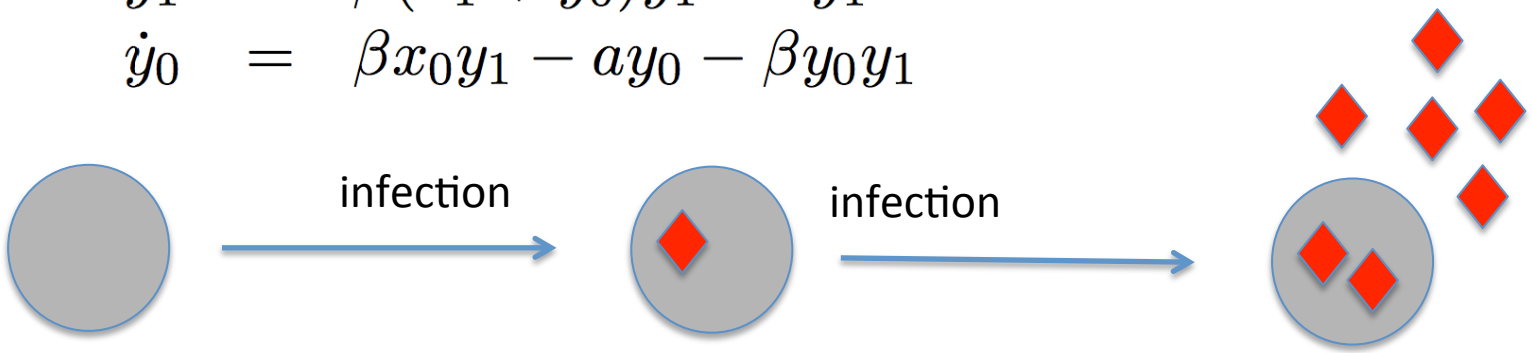


Single infection / slow replication kinetics



Add multiple infection, and assume that multiple infection can overcome anti-viral state

$$
\begin{aligned}
\dot{x}_1 &= rx_1\left(1-\frac{x_1+x_0+y_1+y_0}{K}\right)-dx_1+gx_0-\beta x_1y_1-\gamma x_1y_1\\
\dot{x}_0 &= \gamma x_1y_1-gx_0-\beta x_0y_1-dx_0\\
\dot{y}_1 &= \beta(x_1+y_0)y_1-ay_1\\
\dot{y}_0 &= \beta x_0y_1-ay_0-\beta y_0y_1
\end{aligned}
$$



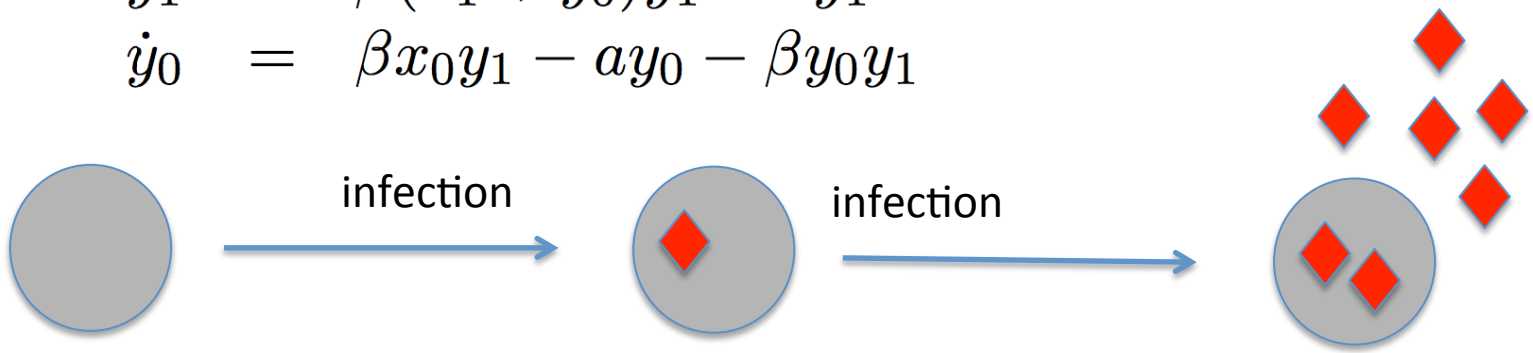
cell in anti-viral state

no virus production due to anti-viral state

multiple infection overcomes anti-viral state, leading to productive infection

Add multiple infection, and assume that multiple infection can overcome anti-viral state

$$
\begin{aligned}
\dot{x}_1 &= r x_1 \left(1 - \frac{x_1 + x_0 + y_1 + y_0}{K}\right) - d x_1 + g x_0 - \beta x_1 y_1 - \gamma x_1 y_1 \\
\dot{x}_0 &= \gamma x_1 y_1 - g x_0 - \beta x_0 y_1 - d x_0 \\
\dot{y}_1 &= \beta (x_1 + y_0) y_1 - a y_1 \\
\dot{y}_0 &= \beta x_0 y_1 - a y_0 - \beta y_0 y_1
\end{aligned}
$$



cell in anti-viral state

no virus production due to anti-viral state

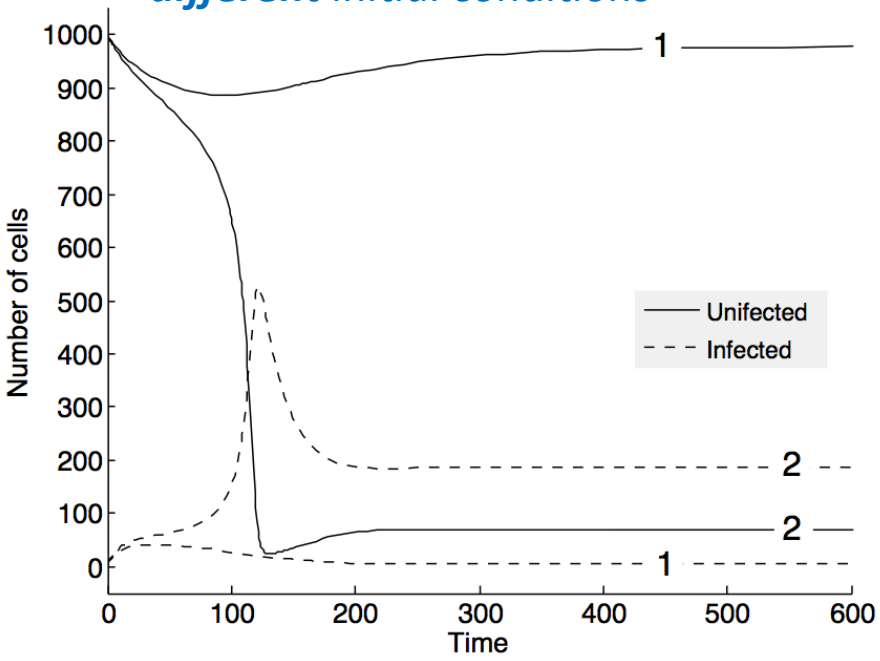
multiple infection overcomes anti-viral state, leading to productive infection

=> Now, model properties depend on initial conditions!!!

Bistability, and extensions to stochastic dynamics

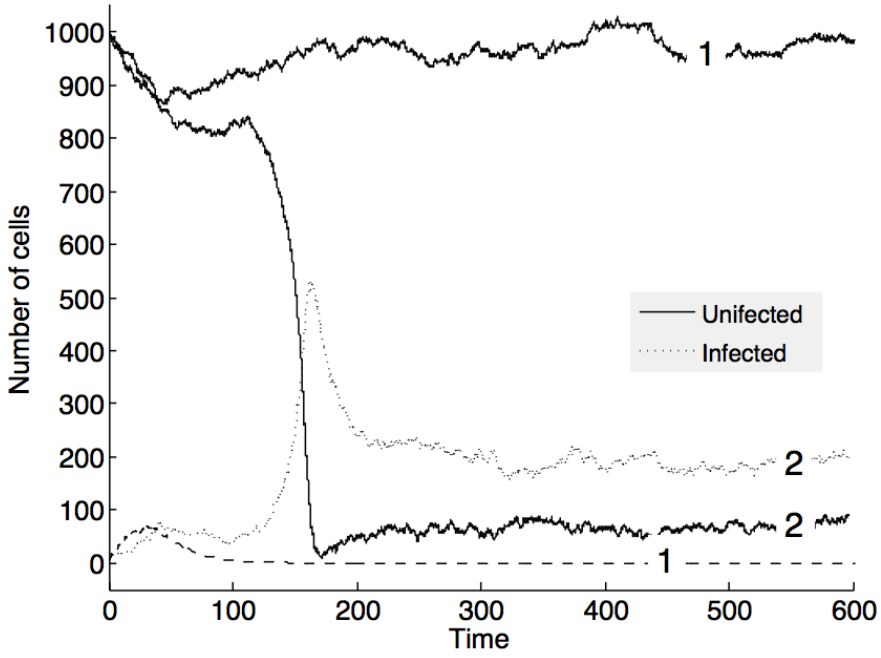
A

deterministic differential equations;
same parameters
***different** initial conditions*



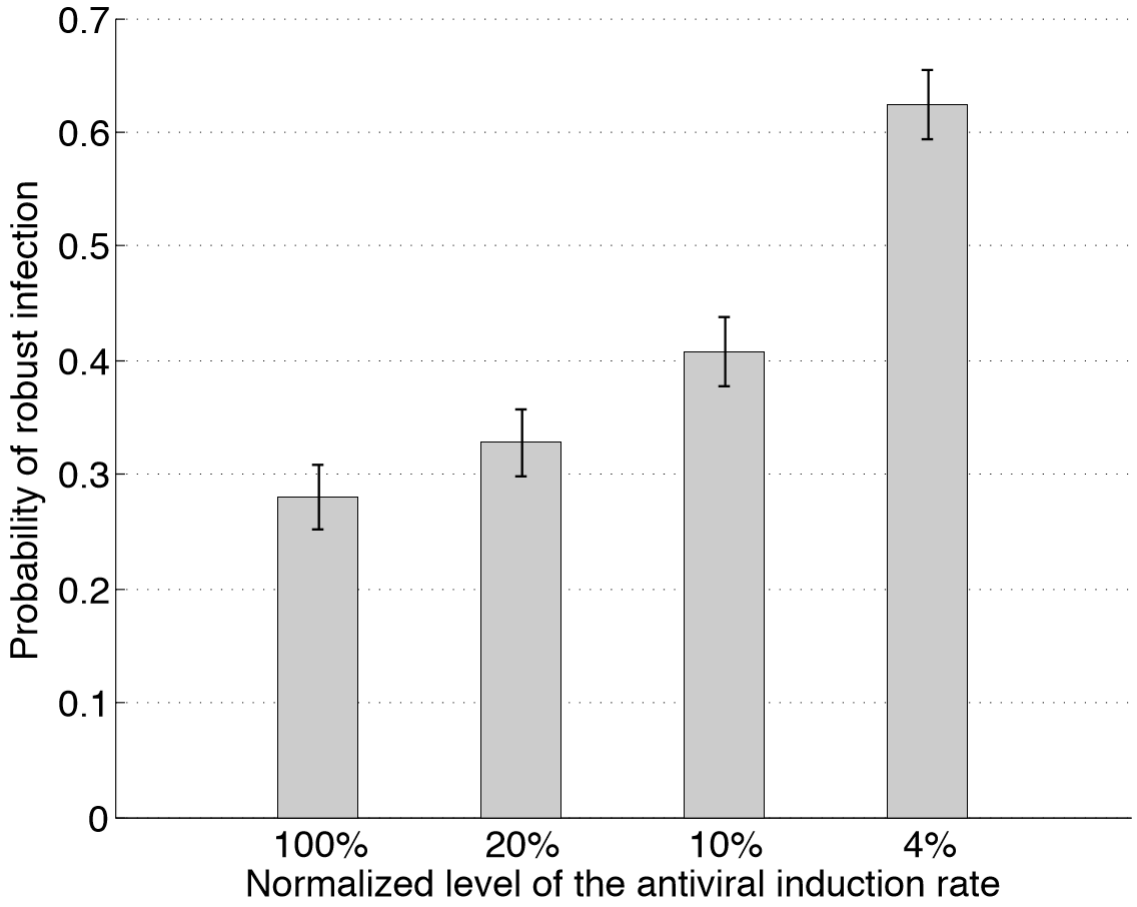
B

stochastic Gillespie simulations of differential equations;
same parameters
***same** initial conditions*

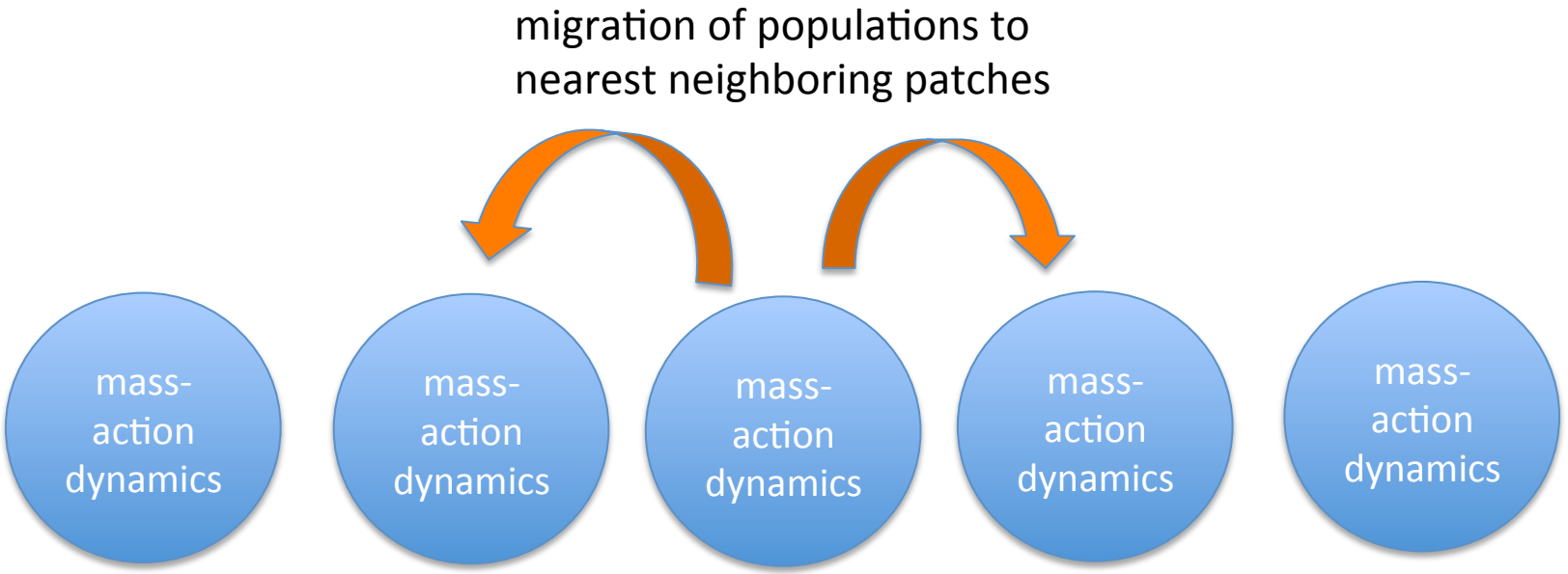


=> a start to explaining how such different dynamic can be observed under indentical conditions, in the same dish

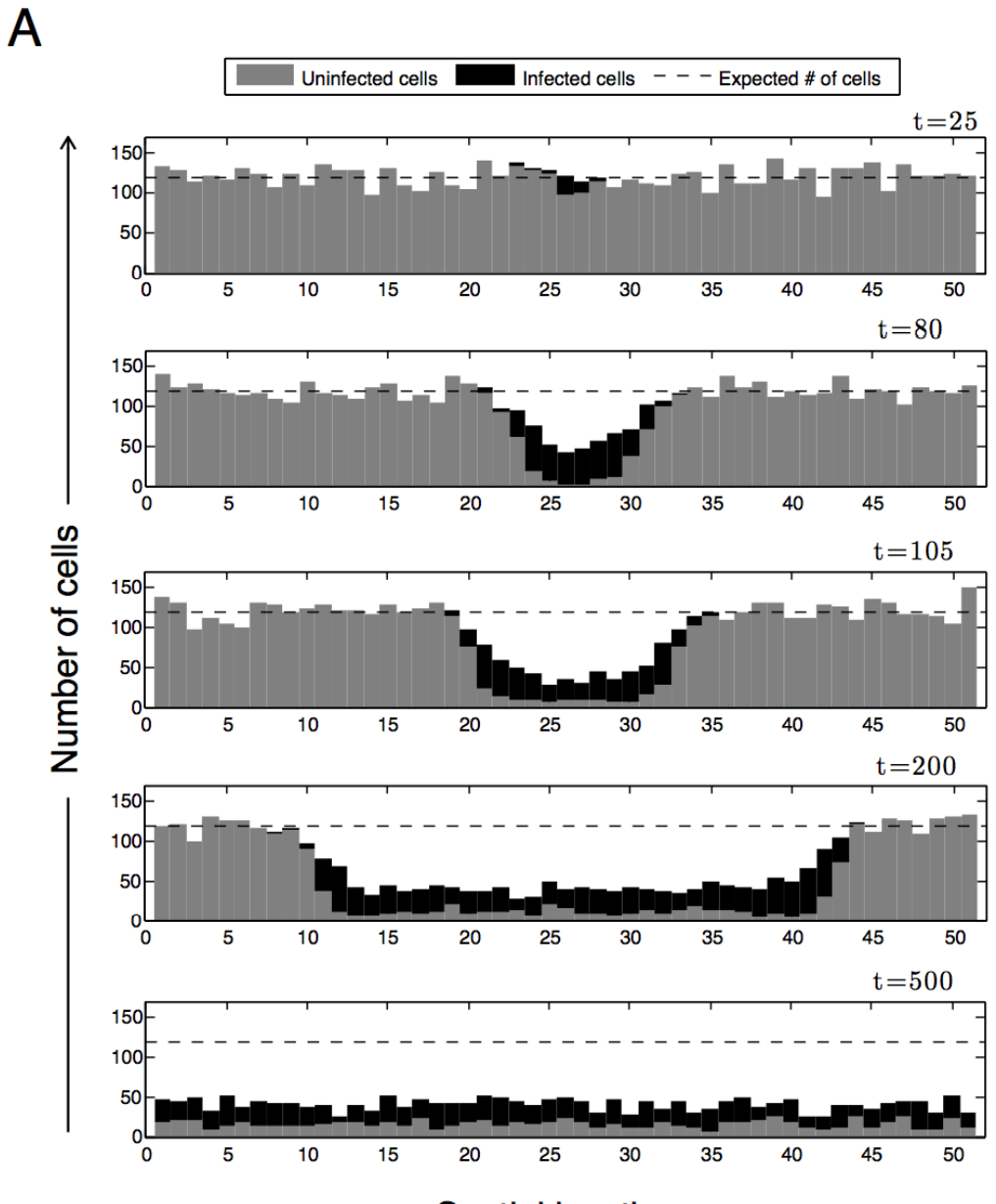
Inhibiting interferon in the model increases fraction of robust infections

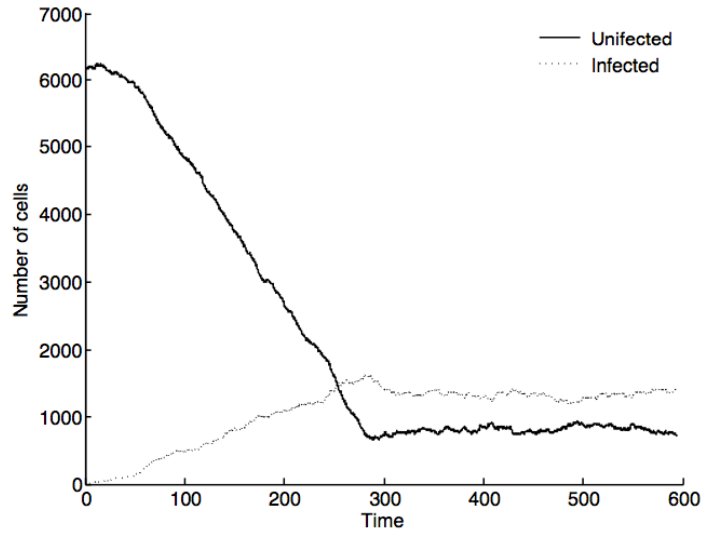


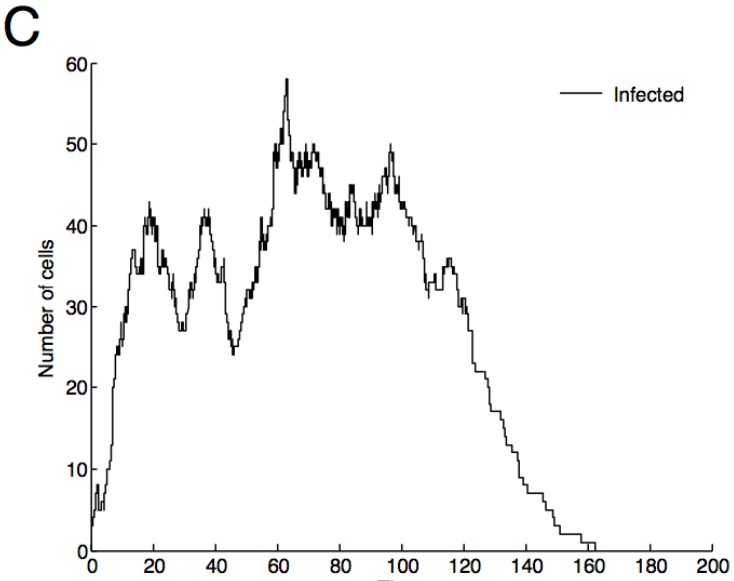
Simplest spatial model metapopulations



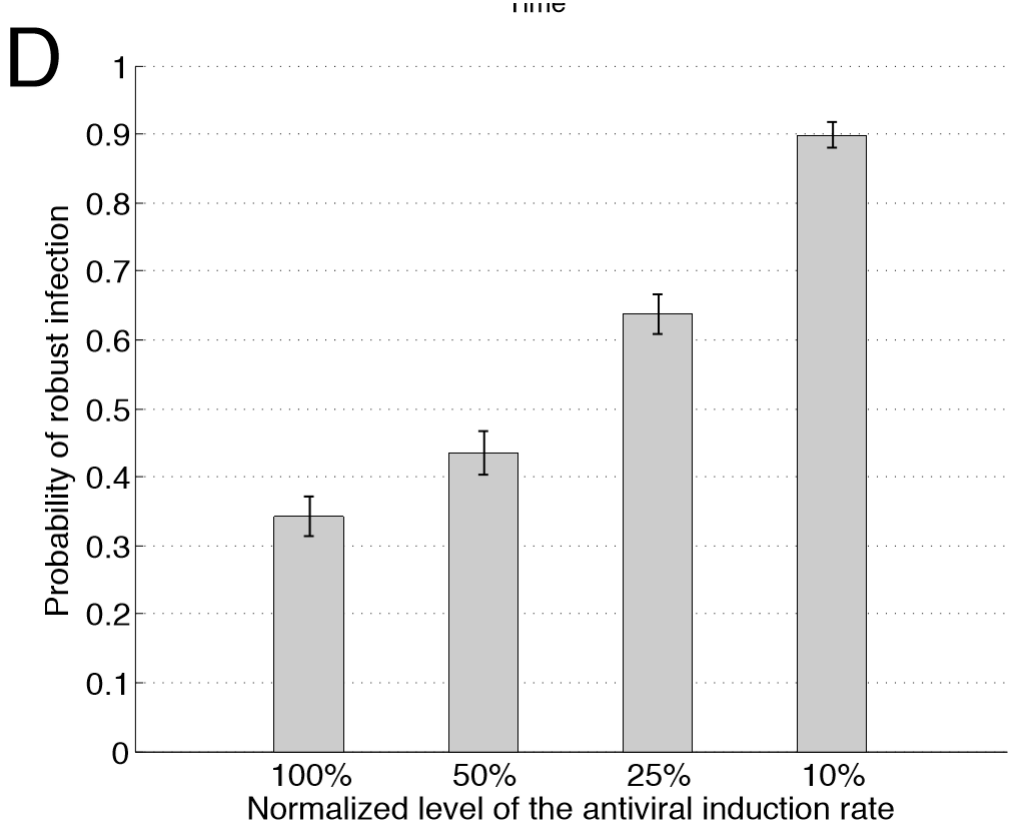
Simulations of metapopulation model



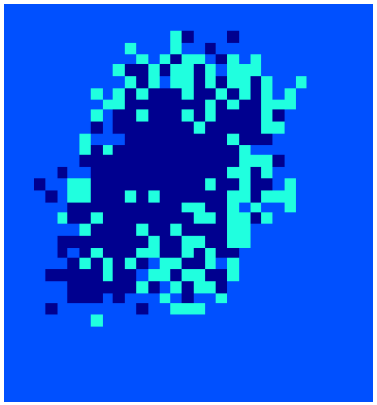


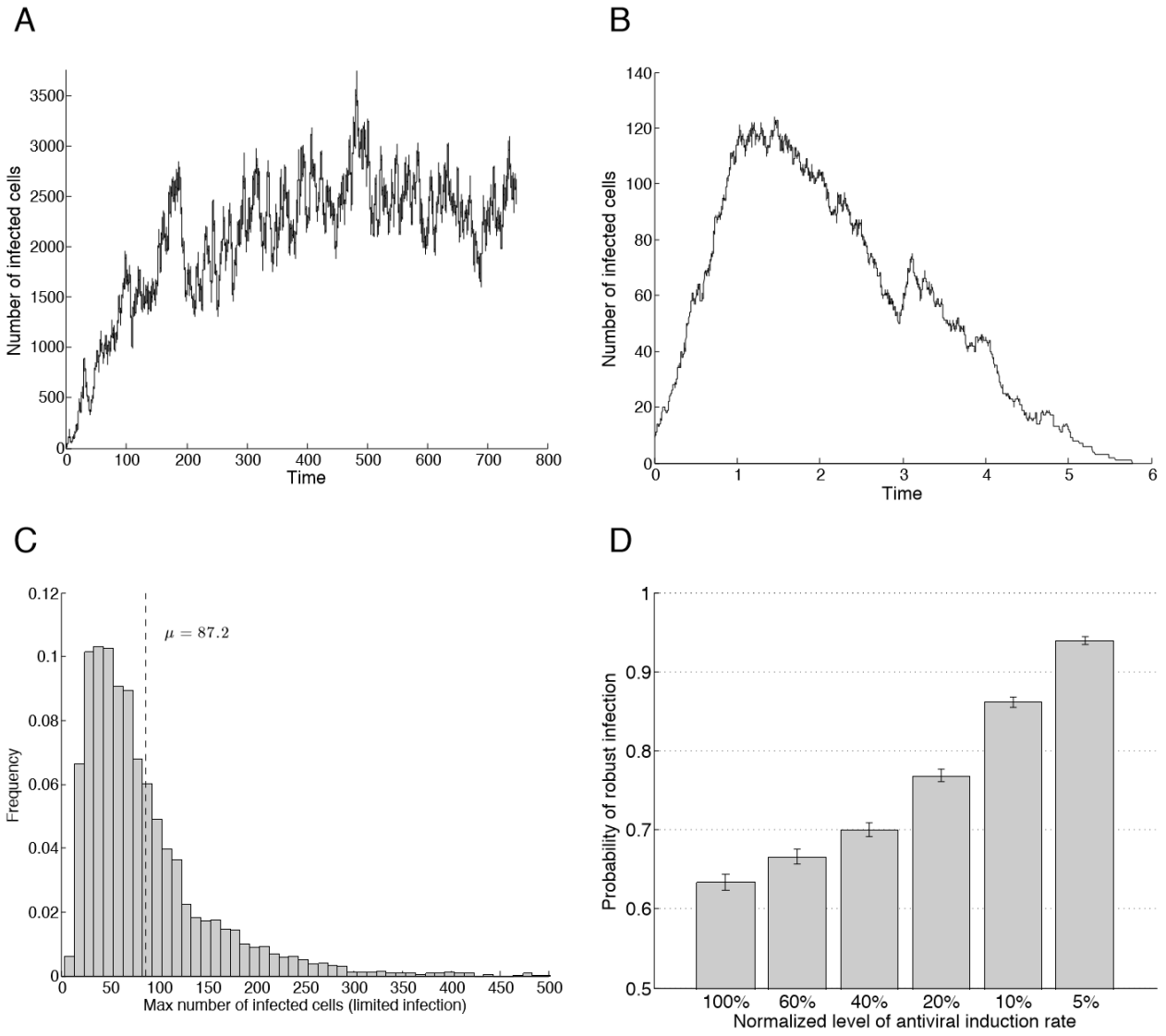


Inhibiting interferon increases fraction of robust infections in metapopulation model as well



Finally back to agent-based model





Model Conclusions

These models tell us that a combination of

(i) Interferon-based anti-viral state induction
(ii) saturation of anti-viral state due to multiple infection

can explain our experimental data

- Mathematical models enabled us to reject experimentally supported hypothesis that IFN-induced anti-viral state alone can explain the data
- The models further enables us to propose an additional mechanism that can contribute to explaining the data
- Further experiments needed for testing => new collaboration with ASU on myxoma virus in a similar setting and beyond

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