Modeling clonal diversity in drug-resistant cancer cell populations

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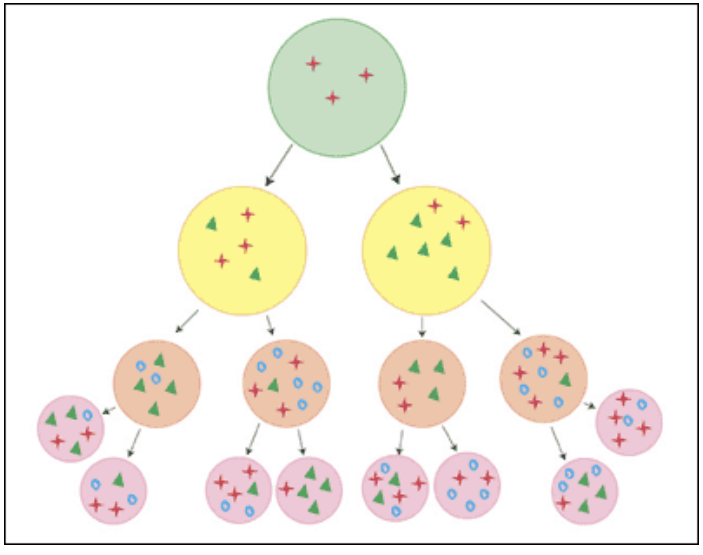
Cancer as an Evolutionary Process

- Variation amongst cells can arise through mutations and other (epi)genetic alterations
- Variation can confer heritable changes in cell fitness

This evolutionary process leads to tumor diversity (seen in sequencing studies, e.g. Ding et al Nature 2012).



Diversity:

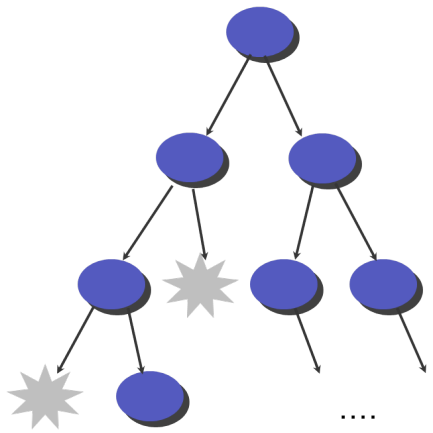
Correlates with disease aggressiveness (e.g Barrett's esophagus, Maley et al 2004)

Tied to emergence and existence of drug-resistant populations, tumor evolvability, etc.

Outline

- Investigations of tumor diversity in a branching process model
- Application to imatinib-resistance in Chronic Myeloid Leukemia
- Application to erlotinib-resistance in NSCLC

Branching model of diversity in tumor growth



Model tumor cell population as a binary branching process. Initially, cells (type 0) give birth at rate a_0 and die at rate b_0.

Define $\lambda_0 = a_0 - b_0 > 0$, $Z_0(t)$ be the number of type-0 cells at time t and suppose $Z_0(0) = 1$.

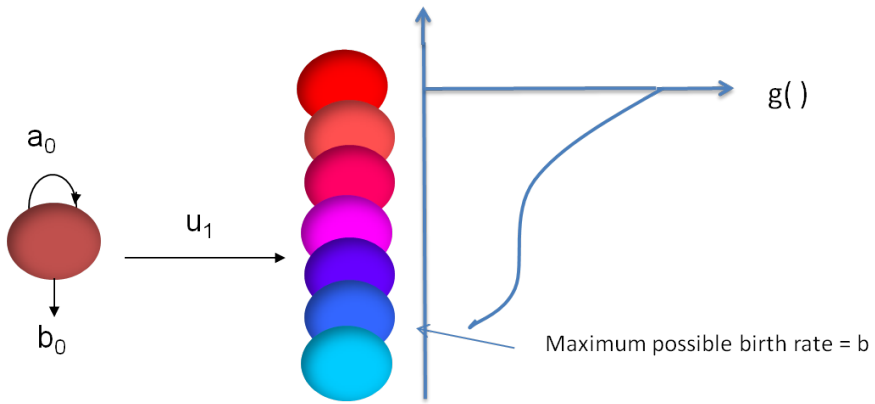
Branching model of diversity in tumor growth

Type-0 cells mutate at rate u_1, creating type-1 cells.

$Z_i(t) \equiv$ type-i cells that have exactly i mutations at time t. Type-i cells mutate at rate u_{i+1}, creating type-$(i+1)$ cells.

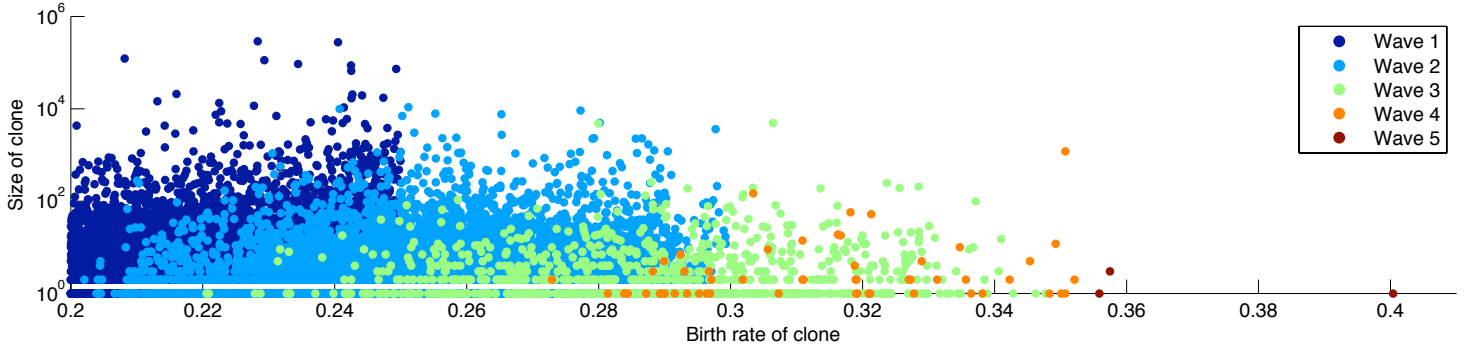
Mutations confer a random additive change ν to the birth rate.

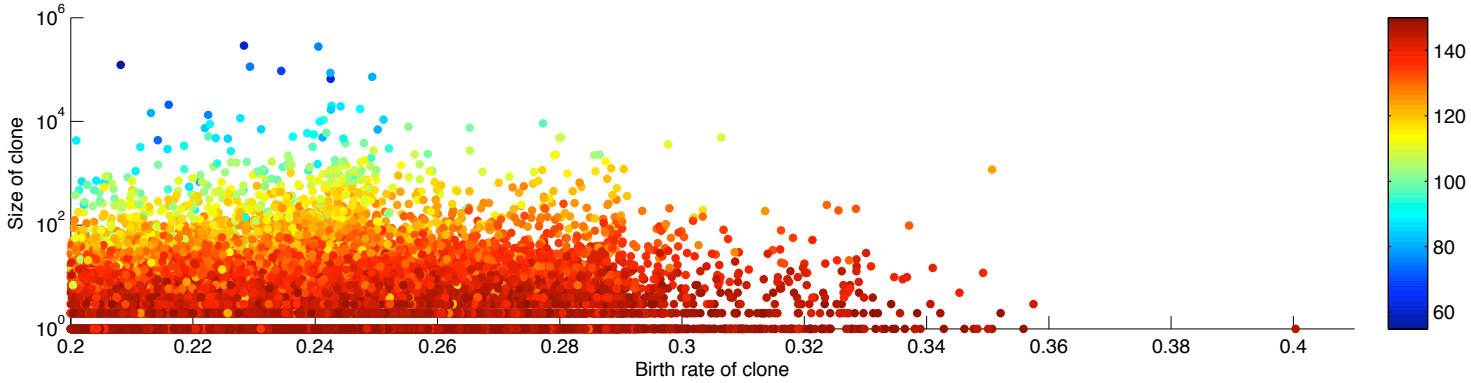
ν has density $g(\cdot)$ on $[0, b]$. b is the largest possible advance



-> investigate diversity properties of Z_1, results generalizable to Z_k.

Intra-tumor diversity generated by model





In this simulation, $\lambda_0 = 0.1, a_0 = 0.2, \nu \sim U([0, 0.05]), u = 0.001$.

How fast does the $Z_1(t)$ population grow?

Theorem

Let $p \equiv b/\lambda_0$. Then

$$t^{1+p} e^{-(\lambda_0+b)t} Z_1(t) \Rightarrow V_1.$$

where V_1 has Laplace transform

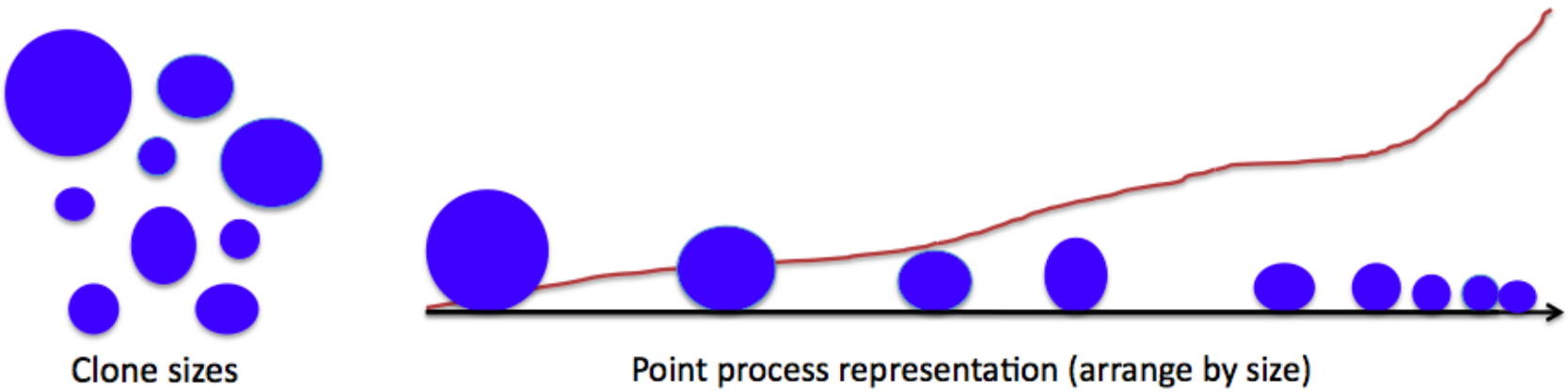
$$\exp\left(-u_1 c_1(\lambda_0, b)\theta^{\lambda_0/(\lambda_0+b)}\right)$$

and $c_1(\lambda_0, b)$ depends on $g(\cdot)$ only at b.

The mean $EZ_1 \sim \frac{u_1 g(b)}{bt} e^{(\lambda_0+b)t}$, so $Z_1(t) << EZ_1(t)$, i.e. the population grows slower than its mean.

Limit V_1 depends on $g(\cdot)$ through endpoint b only.

Point process representation of V_1



Theorem

V_1 is the sum of the points $X_1 > X_2 > \ldots$ in Λ, where Λ is a Poisson process on $(0, \infty)$ with mean measure $\mu(z, \infty) = A_1(\lambda_0, b) u_1 z^{-\lambda_0/(\lambda_0+b)}$, and $A_1(\lambda_0, b)$ is a constant.

Connection to stable laws. The sum of points in V_1 is close to a random walk with stable increments $P(Y_i > x) \sim cx^{-\alpha}$ where $0 < \alpha < 1$.

A diversity measure: Shannon Index

Let p_i be the proportional abundance of clone i in a population.

$$\text{(Shannon Index) } Q \equiv -\sum_{i=1}^{N} p_i \, log \, p_i$$

Point process $V_1 = \sum_{i=1}^{\infty} X_i$, and $X_1 \geq X_2 \geq X_3 \ldots$. Let $p_i \equiv X_i / V_1$.

$(p_1, p_2, \ldots) \sim PD(\alpha, 0)$ (Poisson-Dirichlet)

Pitman and Yor 1997, Perman et al 1992

$$E[Q] = E\left[-\sum_{i=1}^{\infty} p_i \log p_i\right] = -\frac{1}{\Gamma(\alpha)\Gamma(1-\alpha)} \int_0^1 \log(u) u^{-\alpha} (1-u)^{\alpha-1} du$$

$$= \sum_{n=1}^{\infty} \frac{(n+\alpha-1)(n+\alpha-1)\cdots(\alpha)}{n! n}$$

Simpson's Index: a measure of diversity

Define Simpson's Index (the probability two randomly chosen individuals are from the same family) for the point process Λ by

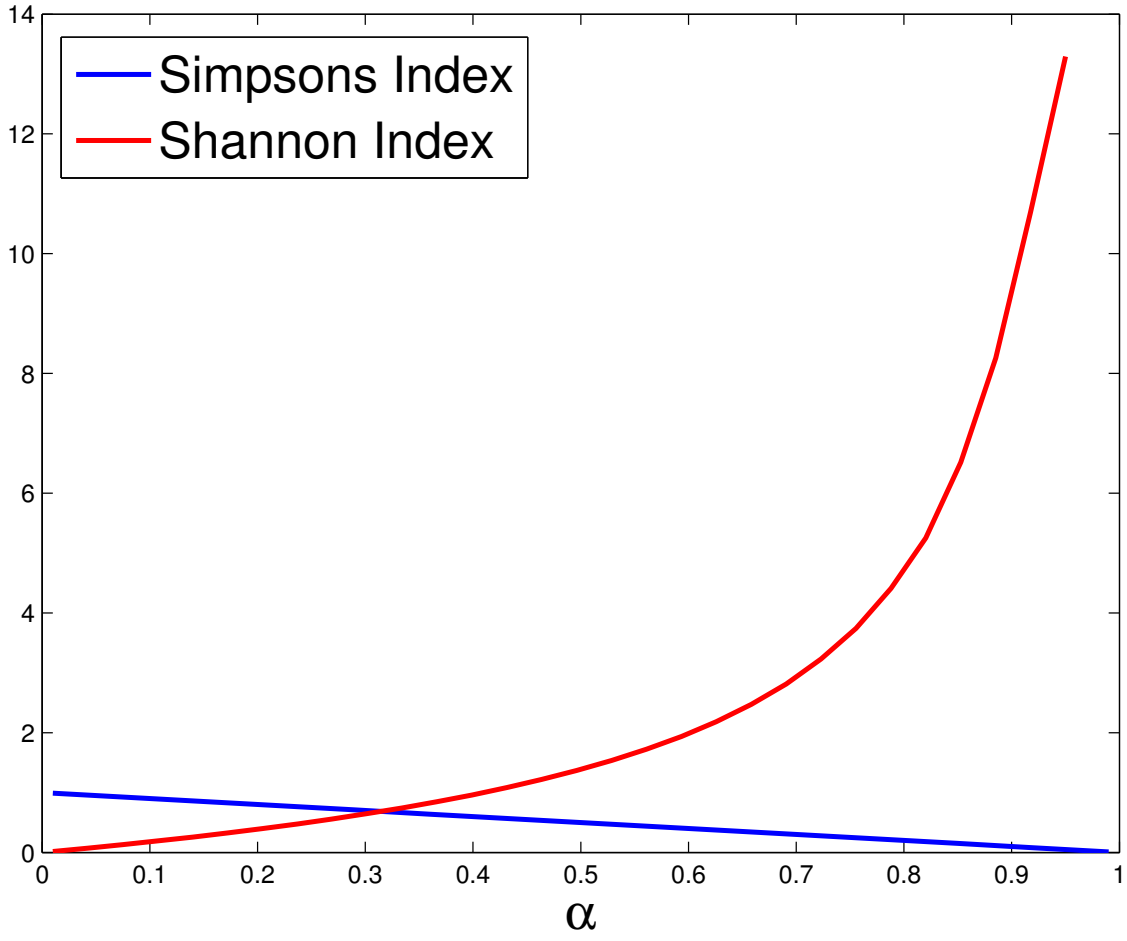
$$R_1 = \frac{\sum_{i=1}^{\infty} X_i^2}{V_1^2}$$

Using the structure of the point process limit and results on self-normalized sums (Fuchs et al 2001), we obtain a simple result:

Theorem (Mean Simpson's Index)

$ER_1 = 1 - \alpha$ where $\alpha = \lambda_0/\lambda_1$.

Comparing diversity measures in Z_1 population



How dominant is the biggest clone?

Let $\theta_n \equiv X_1/S_n$ be the contribution of the largest clone to the sum of the first n largest clones.

$\theta_n^{-1} \Rightarrow W$ and $E[W] = \frac{1}{1-\alpha}$ and $\text{var}(W) = \frac{2}{(1-\alpha)^2(2-\alpha)}$

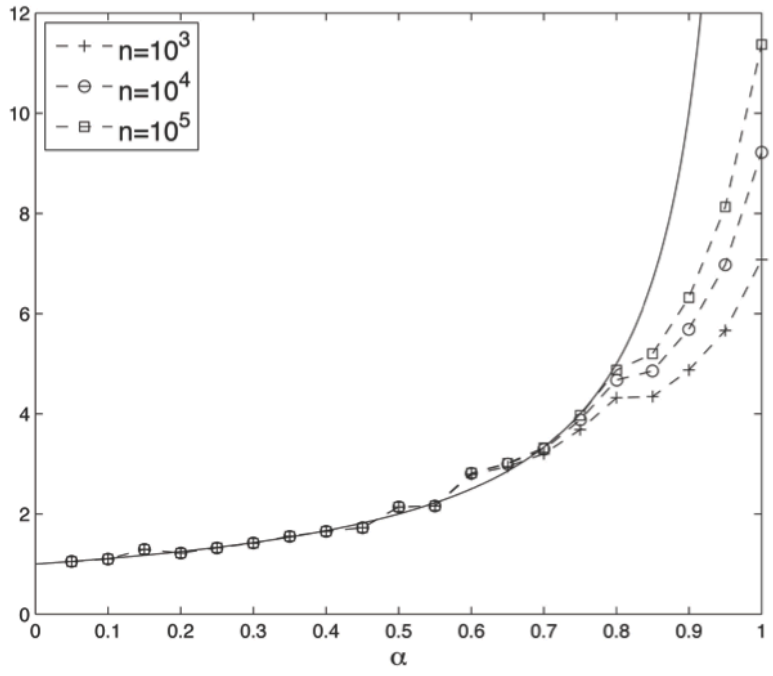


Figure: Convergence of $E[\theta_n^{-1}]$ (dashed) to limit $\frac{1}{1-\alpha}$ (solid)

Summary

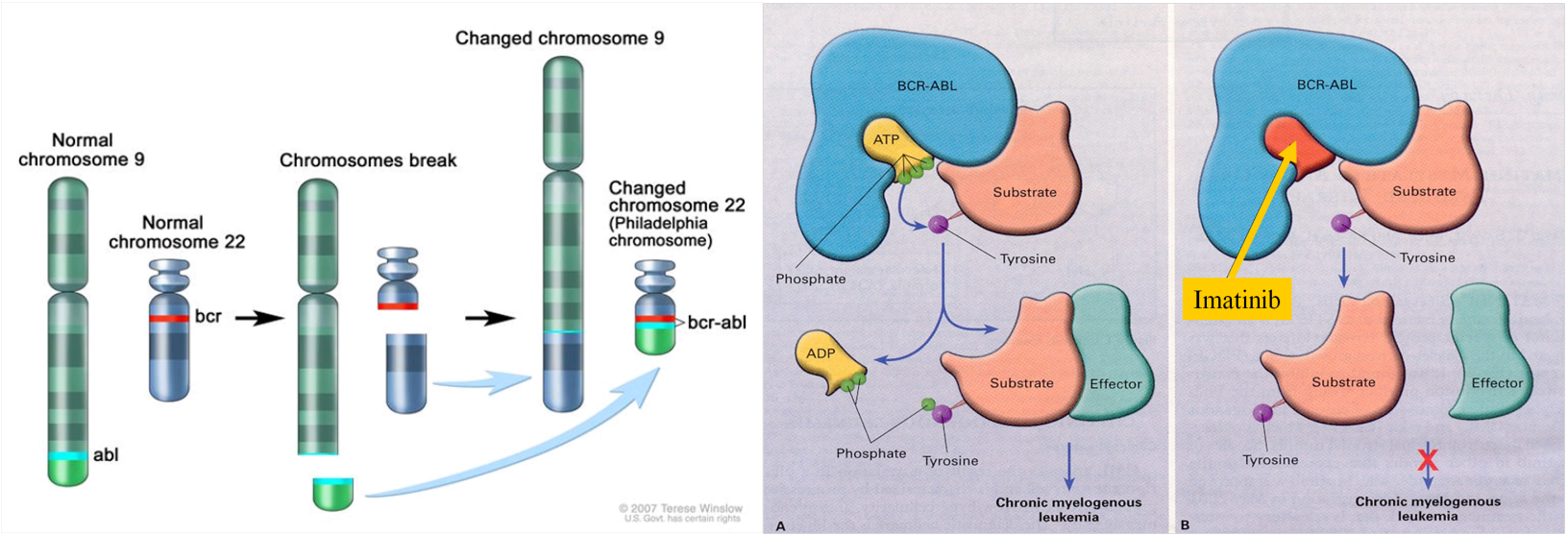
- Growth rate of EZ_k is greater than growth rate of Z_k.
- Asymptotic intra-wave diversity is determined by $\alpha = \lambda_0/(\lambda_0 + b)$, small values of α imply small heterogeneity:
  - Low value of α implies that Simpsons is near 1, i.e., two randomly sampled mutants from wave 1 are likely to be descended from the same mutant, and Shannon Index is near zero (low diversity)
  - If α is small, this implies that the largest clone makes up a large fraction of the total population.

Part II:
Resistance to targeted anti-cancer therapies

Targeted therapies and resistance

- **Targeted therapies**: block growth/spread of cancer by interfering with specific molecules (aka molecular 'targets') involved in tumor growth and progression.
- **However**, despite clinical successes targeted therapies are vulnerable to the evolution of resistance..
- Can we use evolutionary modeling to 1) predict the emergence and characteristics of resistant cell populations prior to treatment? and 2) prevent or delay the emergence of resistance by altering selective pressures (aka treatment schedules)?

Chronic Myeloid Leukemia (CML)



Imatinib, an oral BCR-ABL tyrosine kinase inhibitor (TKI), can induce remission in a large percentage of CML patients.

Application to Chronic Myeloid Leukemia (CML)

Many point mutations confer resistance to imatinib. Dasatinib and nilotinib overcome some imatinib-resistant mutations.

Limitations on testing sensitivity make it difficult to detect low-frequency resistant populations existing at the start of treatment.

Would like to characterize pre-existing resistant populations (e.g. extent, diversity) in order to determine effective first-line treatment protocols.

An Evolutionary Model of Resistance

- Sensitive CML stem cell pop. at time t: $Z_0(t) = V_0 e^{\lambda_0 t}$. V_0 is exponential random variable with mean a_0/λ_0.
- Resistant stem cells created with probability u each time a sensitive cell divides. Death rate b_0, birth rate $a_0 + X$.
- Values of X have equal probability and correspond to growth rates of each of the 11 most common resistant types. (Given on next slide)

Focus on Wave-1 of mutants (Z_1) since parameters indicate small likelihood of Z_2 arising before detection.

Growth Rates of Resistant Mutants in CML

In vivo growth rates adapted from in vitro measurements of sensitive cells and 11 resistant types (B. Skaggs, C. Sawyers)

Cell Type	Birth Rate	Resistant to
T315I	0.0088	all
E255K	0.0085	imatinib
Y253F	0.0082	imatinib
p210	0.008	**
E255V	0.0078	imatinib
V299L	0.0074	dasatinib
Y253H	0.0074	imatinib
M351T	0.0072	imatinib
F317L	0.0071	imatinib, dasatinib
T315A	0.0070	dasatinib
F317V	0.0067	dasatinib
L248R	0.0061	imatinib, dasatinib

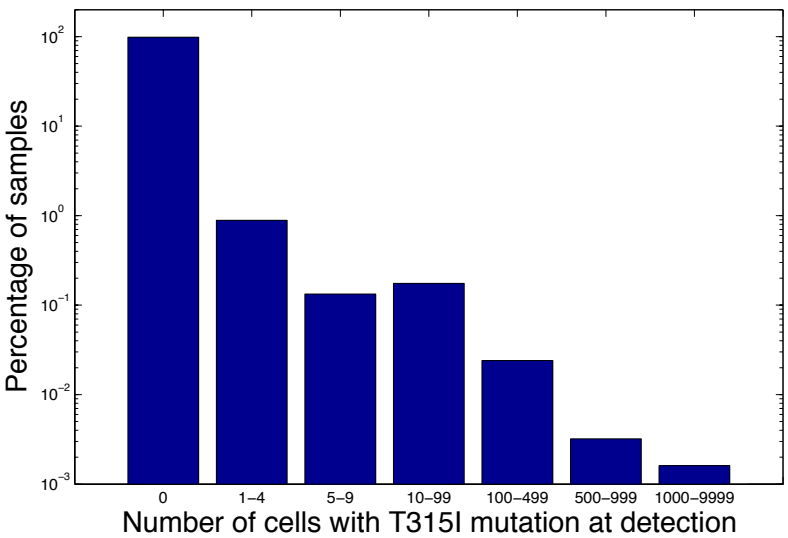
T315I: pan-resistant

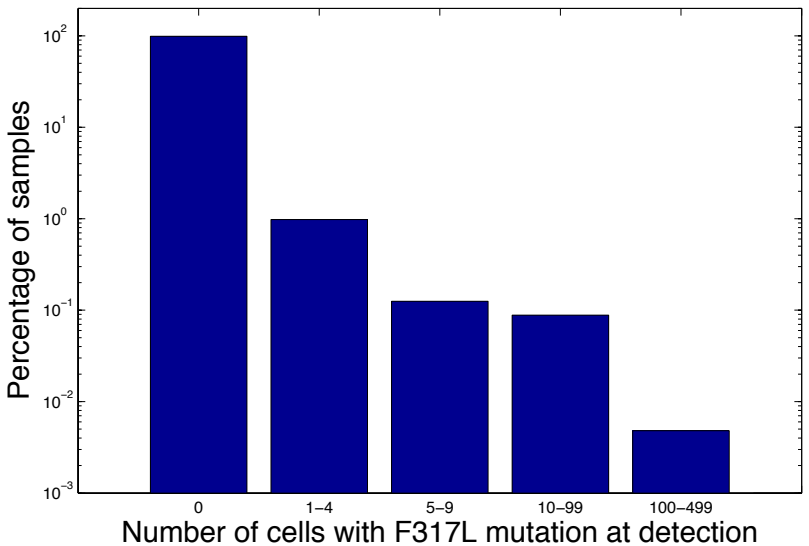
Probability of resistance at time of diagnosis

Time of diagnosis τ_M= when population reaches size M. Normal number of leukemic stem cells at diagnosis $M \approx 10^5$ (Holyoake et al 1999).

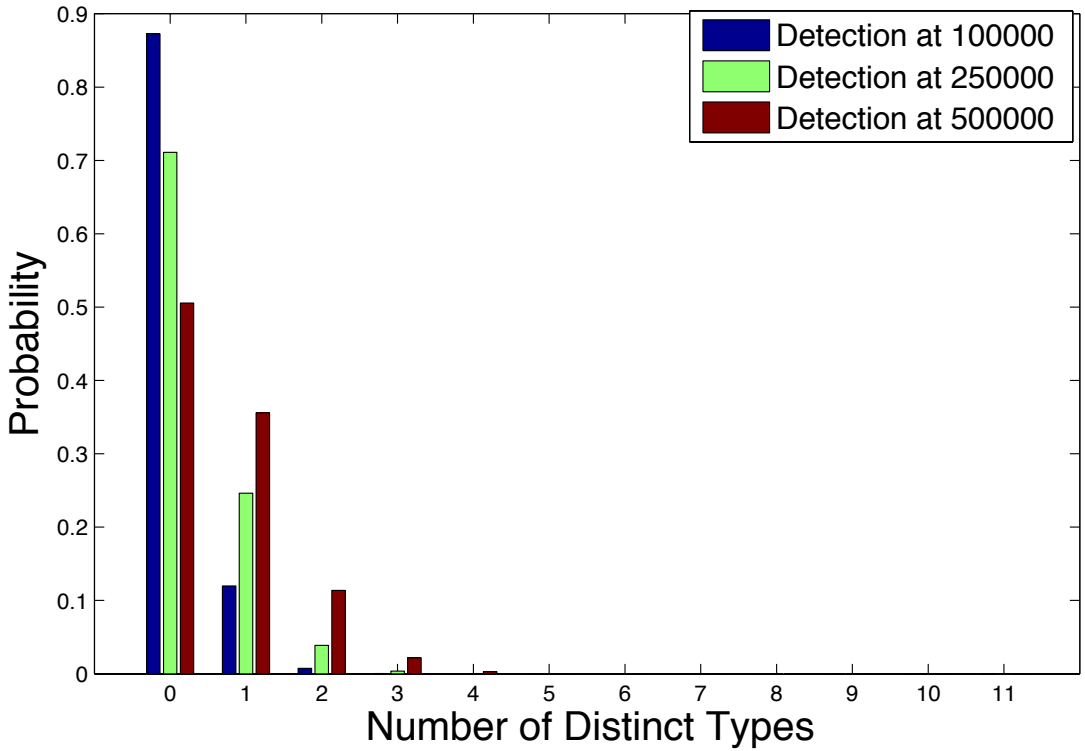
Probability of each mutant existing at diagnosis is roughly 1.2%.

However, clone size distribution may very between mutants.





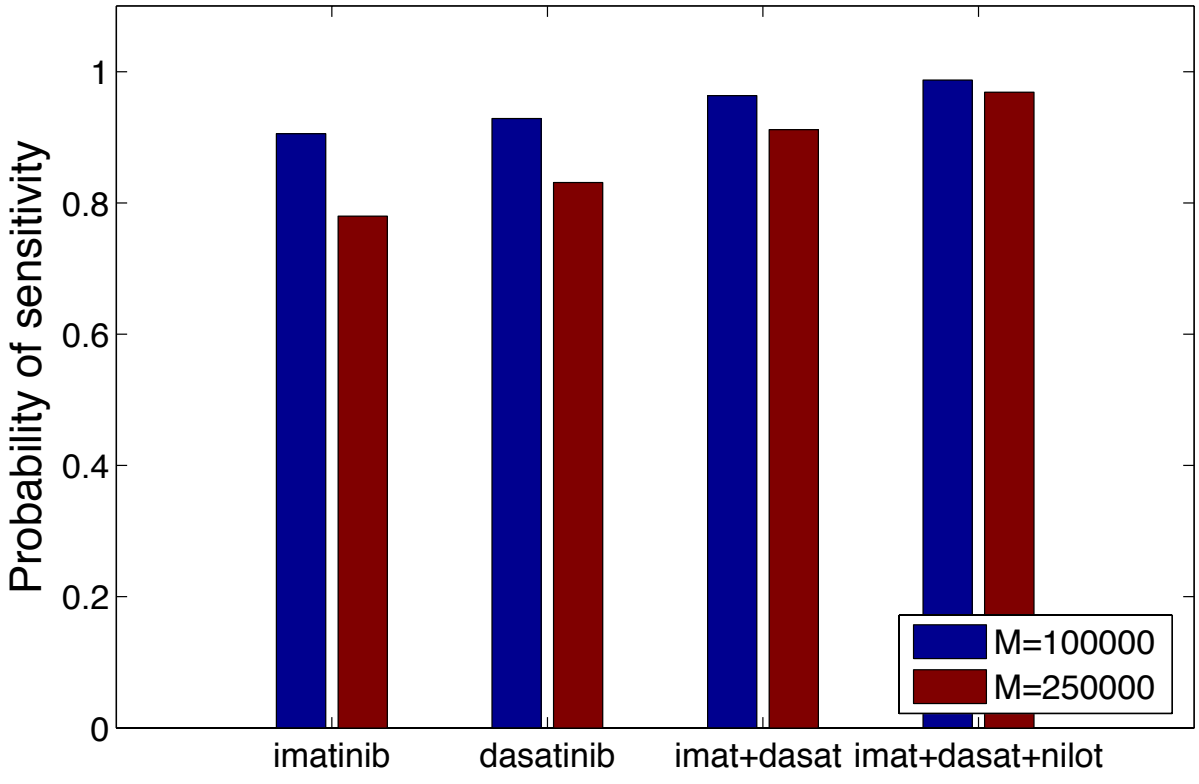
Number of resistant types at the time of diagnosis



At normal detection levels, approx 12-13% of patients have at least one type of pre-existing resistance.

Later detection leads to a more diverse resistant population, with 2-3 distinct types possible.

Evaluating benefits of combination therapy



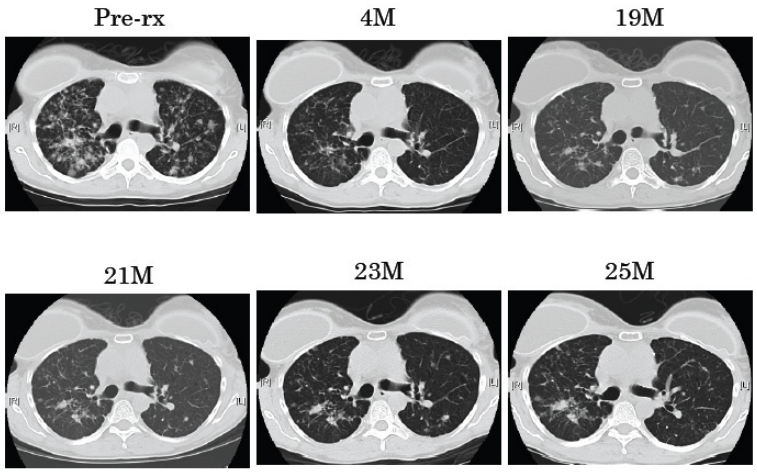
Benefits of combination therapy over monotherapy with imatinib significantly greater for patients with late detection.

Non-small cell lung cancer (NSCLC)

Specific mutations in the Epidermal Growth Factor Receptor (EGFR) associated with sensitivity to targeted drugs such as erlotinib and gefitinib (tyrosine kinase inhibitors).

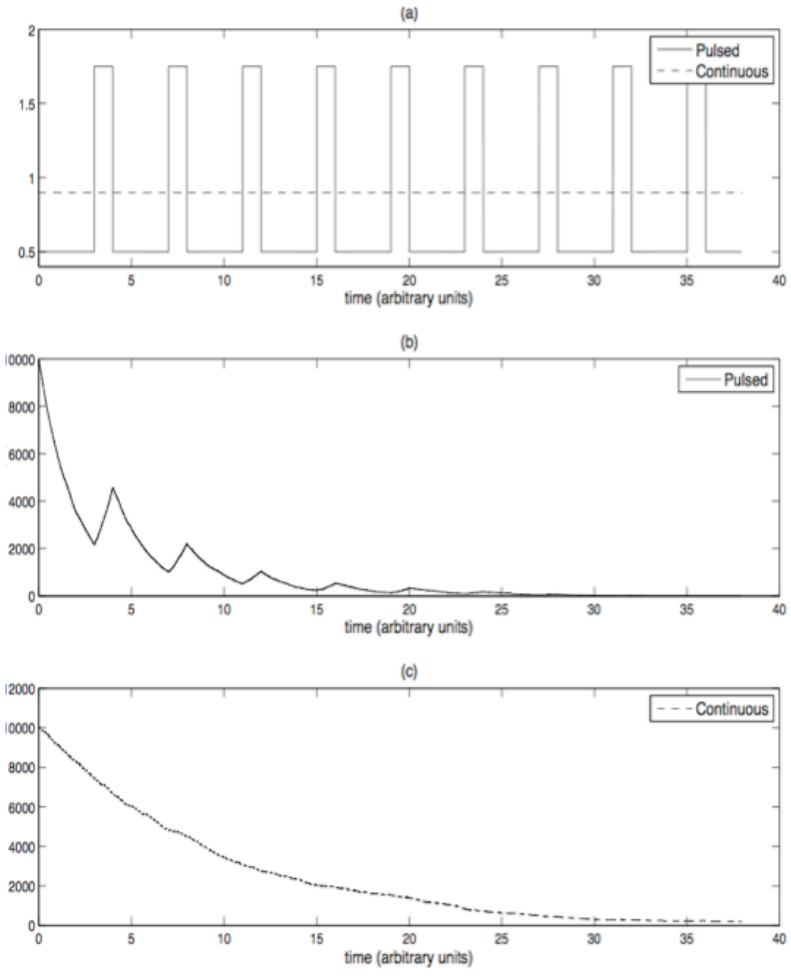
Despite initial response to therapy, 100% of patients develop resistance (usually within $\sim$ 10 months).

Resistance is associated (in approximately 50% of patients) with a single point mutation (T790M) within EGFR. (Pao et al, 2006)



Evolutionary model of resistance during treatment schedules

- Sensitive ($Z_0(t)$) and resistant ($Z_1(t)$) cells are binary branching processes. Initial pop can be mixture of sensitive and resistant cells.
- Resistant cells created with probability u each time a sensitive cell divides (only one resistant type - T790M)
- Birth/death rates of each cell type time-dependent (based on current drug concentration).
- Experimental data used to determine relationship between drug concentration and growth kinetics.



Characterizing model parameters

Isogenic sensitive/resistant pair of NSCLC lines developed with and w/o T790M mutation (by W. Pao, J. Chmielecki)

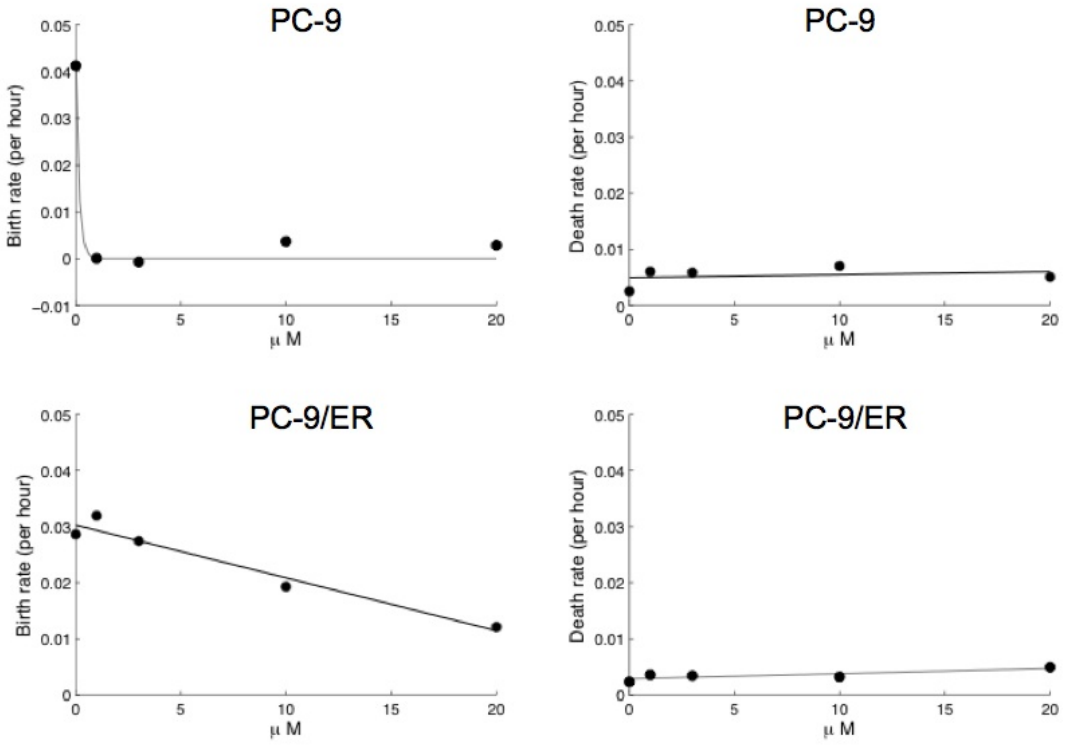
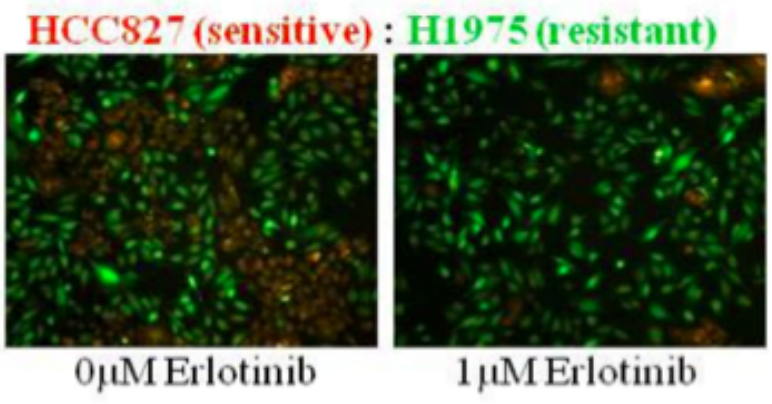


Figure: Growth and death rates of sensitive (PC-9) and resistant (PC-9/ER) cells vs. erlotinib concentration.

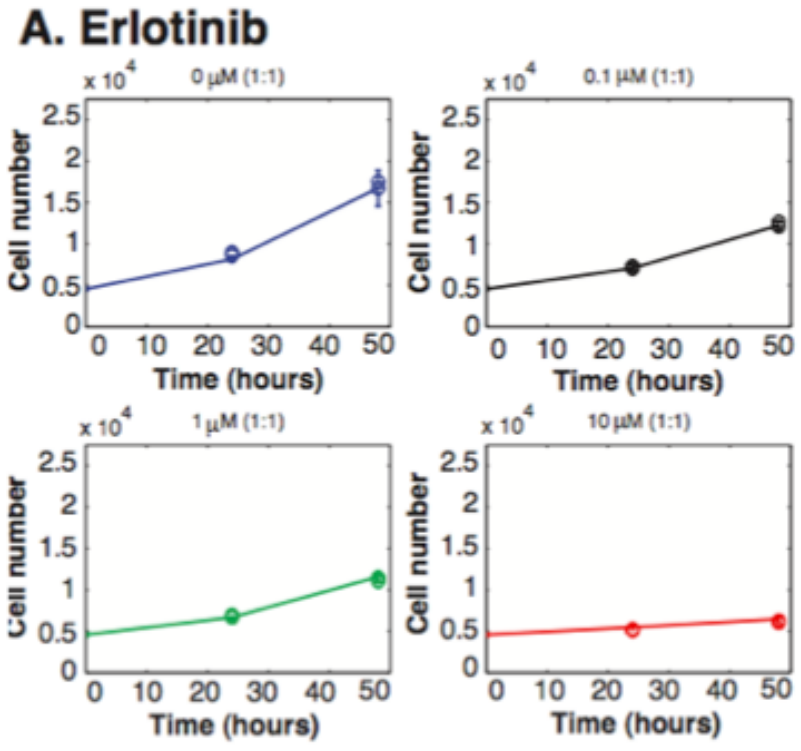
Model testing and validation

Quantitative imaging using Cellomics Arrayscan

Fluorescence tagging of sensitive and resistant cells enables detailed quantitative measurements over time (with spatial resolution)



Shannon Mumenthaler (USC)
Parag Mallick (Stanford)



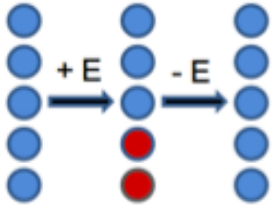
Model predictions – circles, Experiment – line. Admixtures 1:4, 4:1, 1:9 also performed (not shown). Mean error < 10%

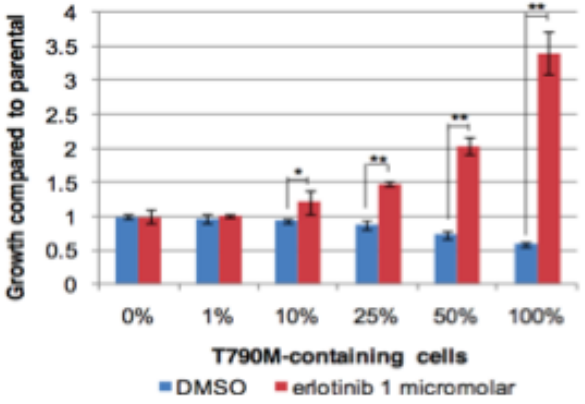
Model testing and validation

Resensitization of a polyclonal resistant population:

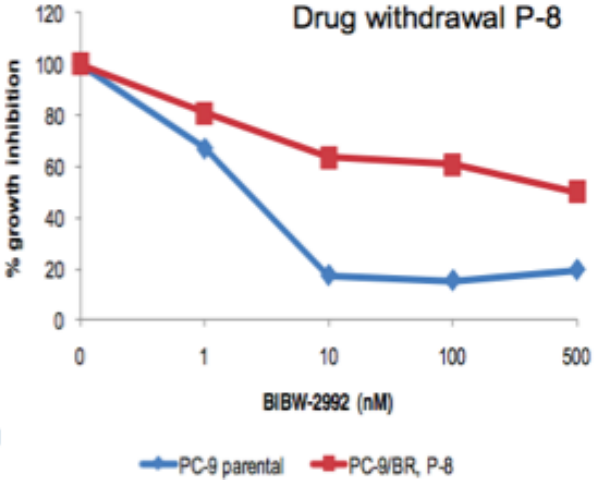
- Modeling prediction: ~ 35-40 days needed in absence of drug for a population of cells initially 87.5% resistant to become <1% resistant.

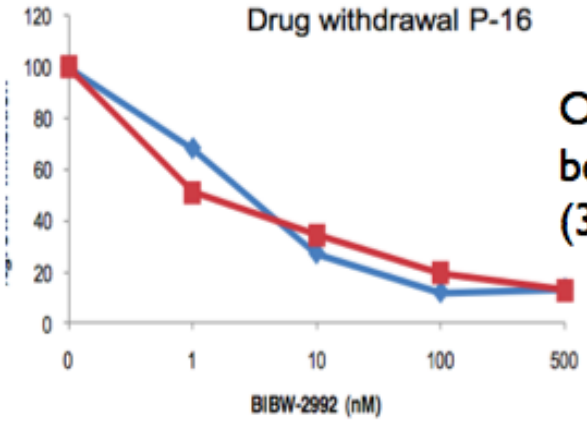
Heterogeneous population:
only some cells have T790M





Juliann Chmielecki, William Pao (Vanderbilt)



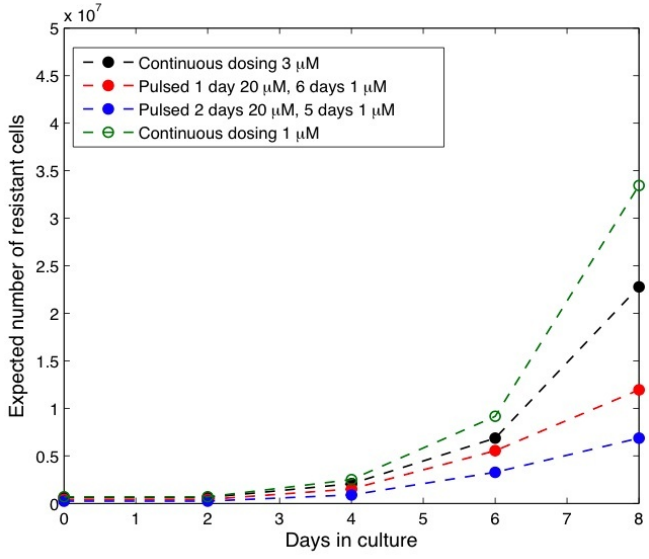


Observed resensitization between 8-16 passages (3 days/passage)

Optimized treatment schedule that delays resistance

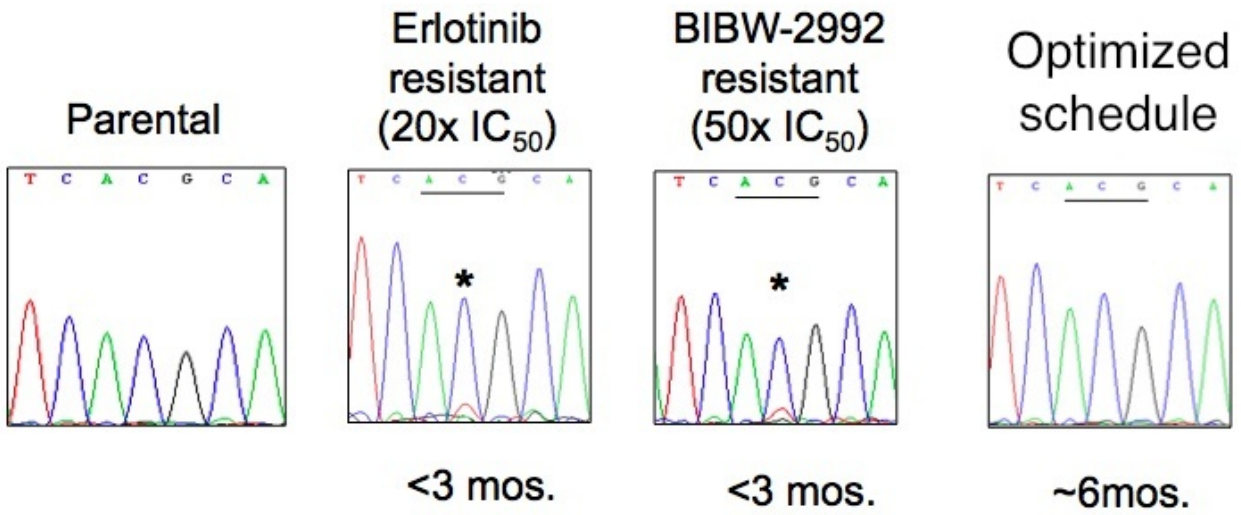
- Current FDA approved schedule: continuous daily dose eliciting 3uM Cmax concentration in plasma.
- Validate model, evaluate a range of possible dosing strategies and search for strategies that maximally delay resistance.
- We identify an alternate tolerated schedule that should delay resistance:

  Oral intake eliciting 20uM pulse 1/wk (or more potent inhibitor)+1 uM/day schedule.

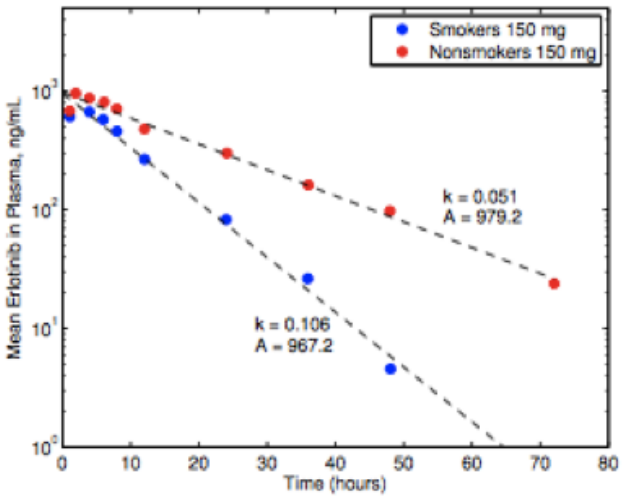


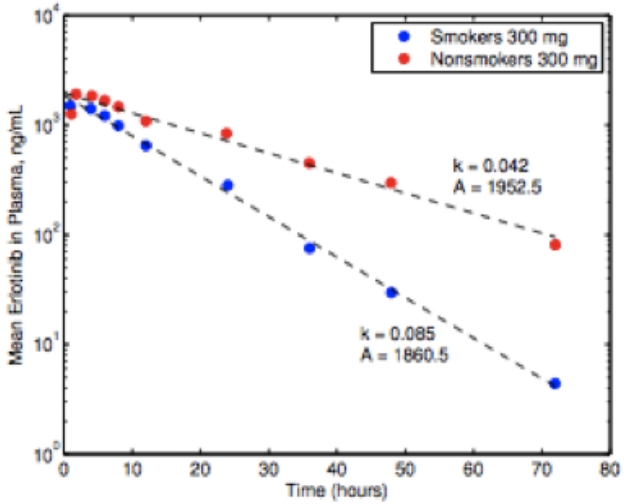
Validation in cell lines

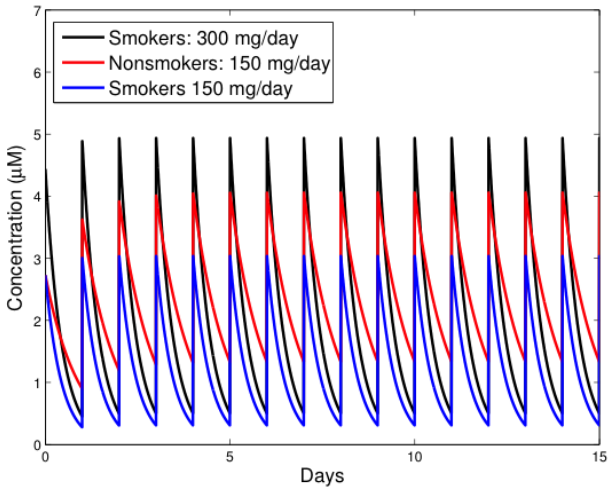
Hypothesis: High dose pulse (BIBW-2992) 1 day/wk + Very Low dose Erlotinib 6 days/wk will result in longer time to develop resistance than the currently-used continuous dosing strategy.



Effects of pharmacokinetic variability on resistance







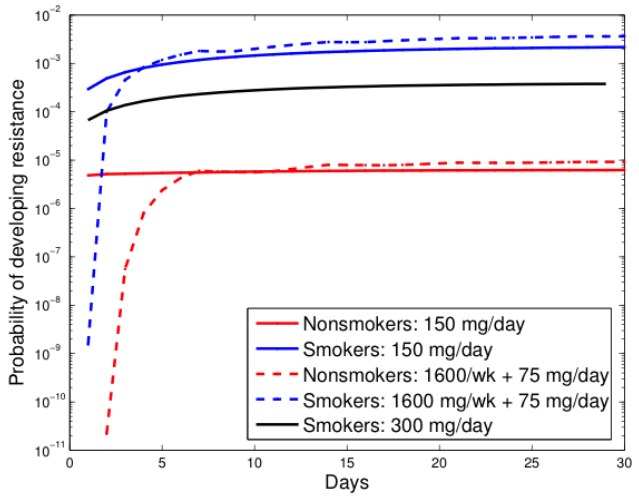
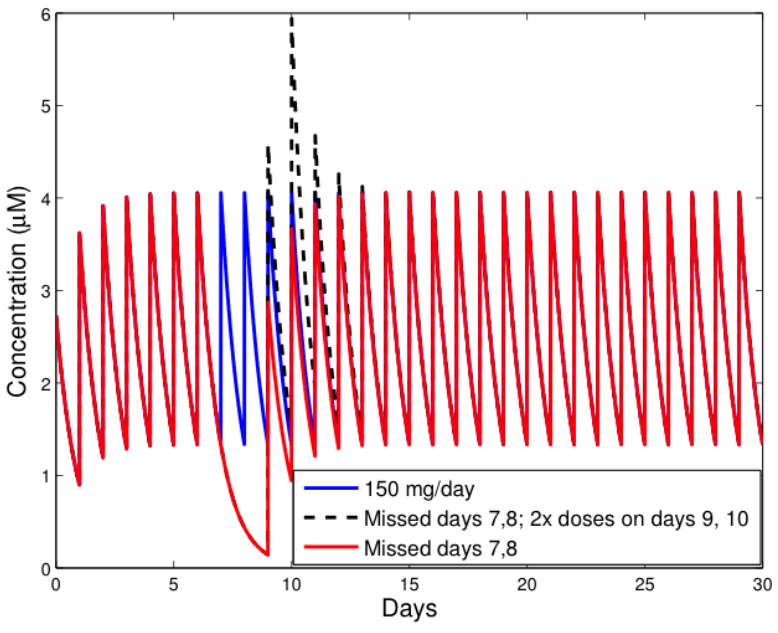


Figure: PK trial data: Hamilton et al, CCR 2007

Effects of non-compliance on resistance



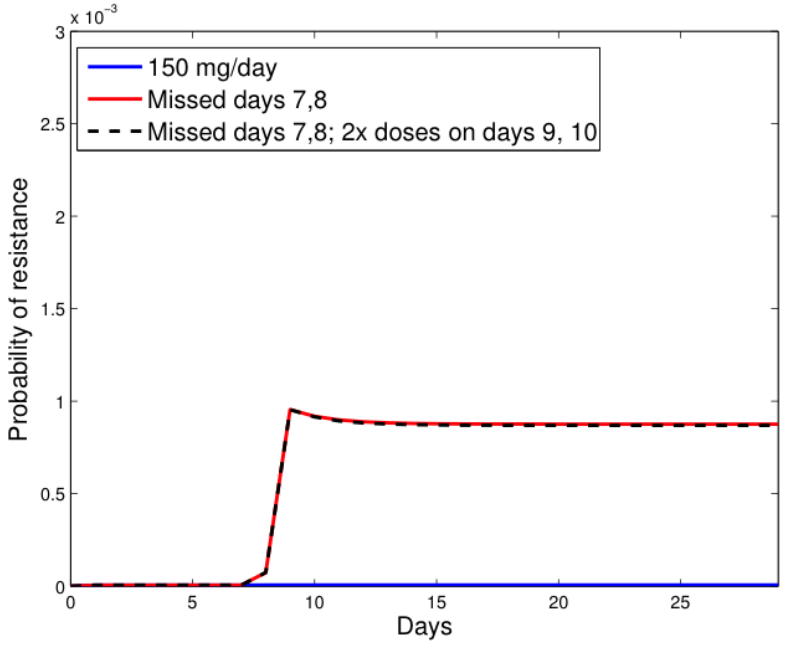


Figure: Effects of missed and makeup doses

Summary

- Branching model of tumorigenesis with random mutational fitness effects drawn from continuous distribution.
- Asymptotic diversity in resistant population is determined by $\alpha = \lambda_0/(\lambda_0 + b)$, small values of α imply small heterogeneity. (results also obtained for later waves, inter-wave heterogeneity)
- Applications to CML and NSCLC may provide useful information for better understanding of inter-patient variability in response, and the effects of dose modification and non-compliance on development of drug resistance.

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