Cancer cell plasticity
Drug persistence and Beyond

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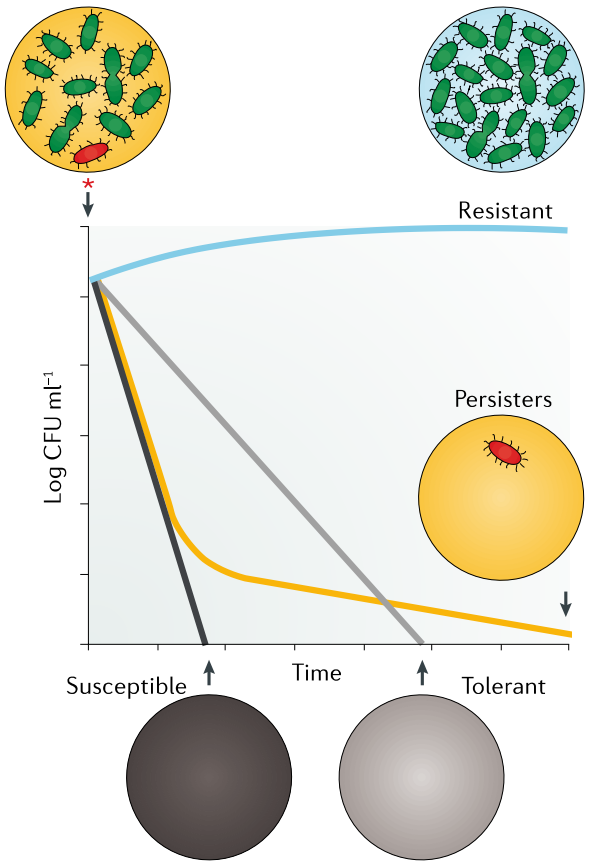
Kessler, David A., and Herbert Levine. "Phenomenological approach to cancer cell persistence." *Physical Review Letters* 129.10 (2022)

Park, J. T., & Levine, H. (2025). Mathematical characterization of drug-induced persistence in cancer. *bioRxiv*, 2025-01. (new version, in preparation)

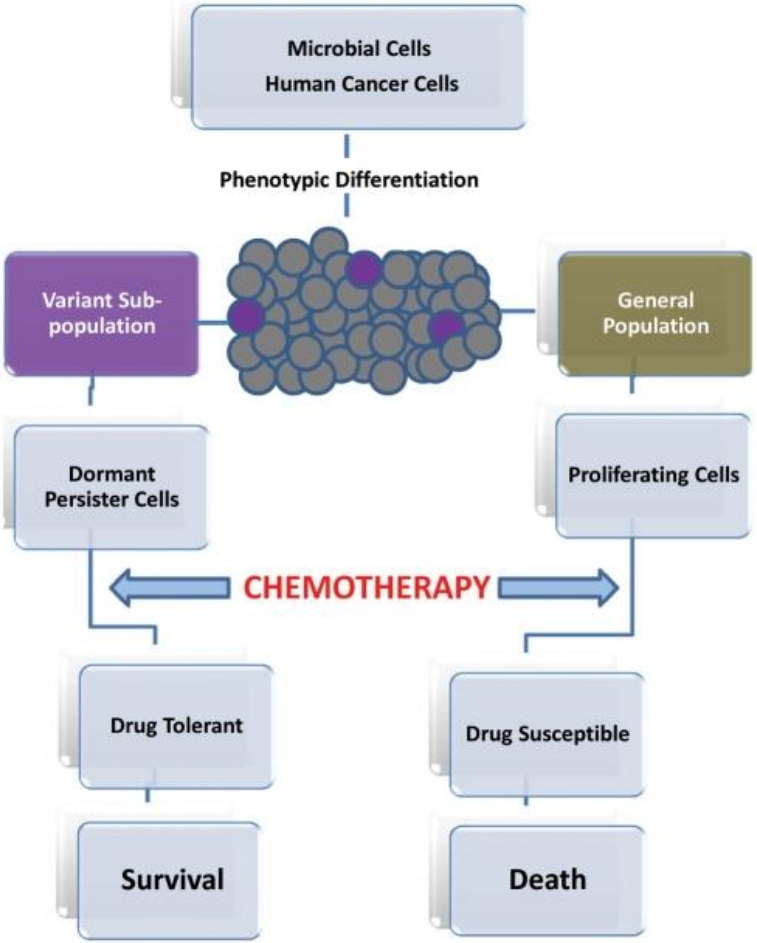
Bacterial Persistence



- Persisters in the presence of antibiotics identified long ago by Biggers
- Classic two decay behavior; second decay may be slower death rate or just transition back to sensitive
- Distinct from drug resistance in that it is reversible

Fisher, R., Gollan, B. & Helaine, S. Persistent bacterial infections and persister cells. Nat Rev Microbiol 15, 453–464 (2017)

Simplest picture



From a mathematics point of view

- Pre-existing multistable network
- Persisters are selected by drug
- Analogous to resistance mutants, modeled by Luria-Delbruck distribution, except for possible reversion

We do not think this type of model is sufficient for the cancer system; instead we will introduce a continuous distribution of persistence, and allow their formation to be drug-dependent

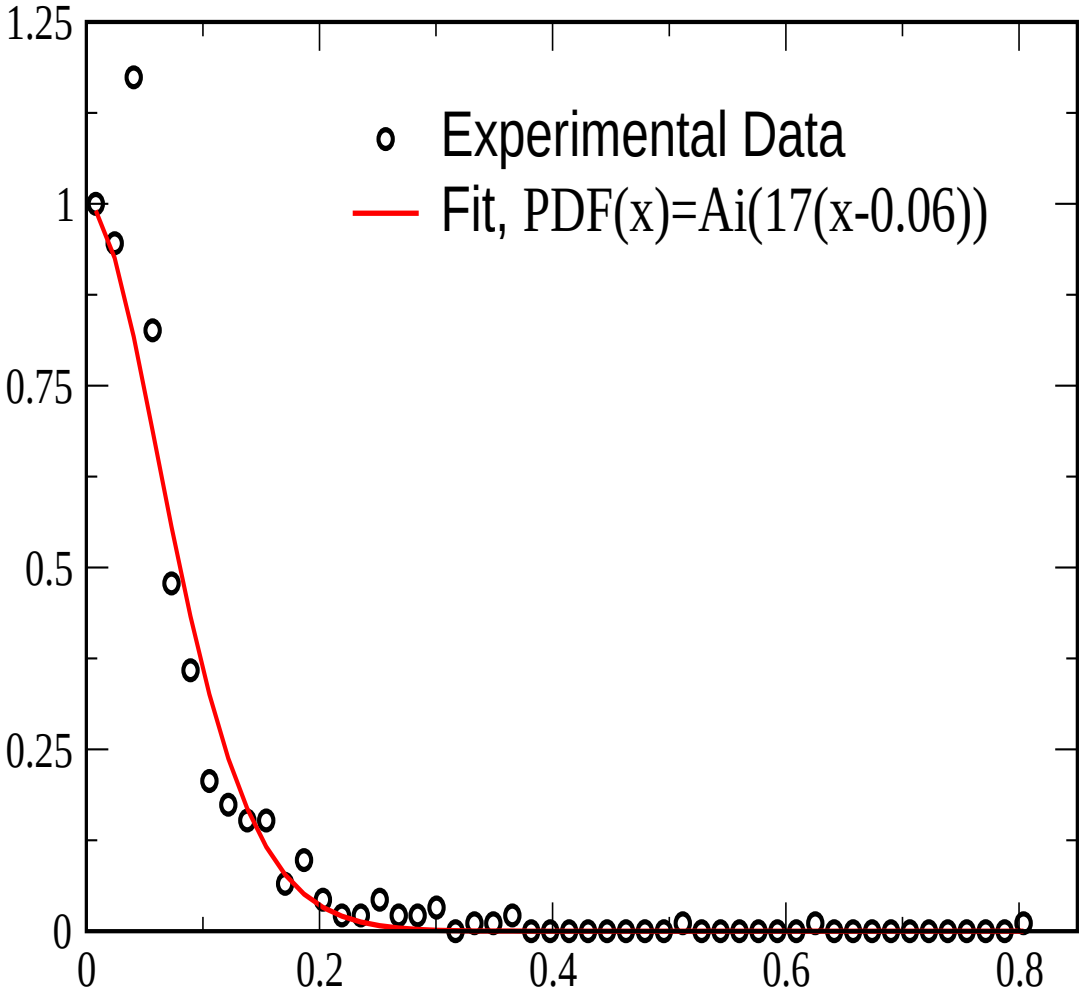
Degree of persistence seems to be a quantitative trait

Started with Experiment

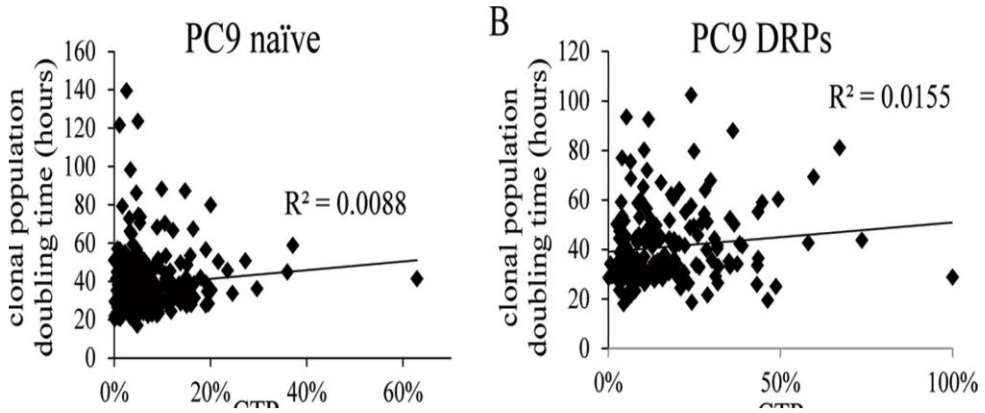
Jacob Berger et al. "IRS1 phosphorylation underlies the non-stochastic probability of cancer cells to persist during EGFR inhibition therapy." *Nature Cancer* 2.10 (2021): 1055-1070.

Persisters are defined as cells which survive past day 7. What is plotted here is the percentage of persisters for a series of **single cell clones**

What accounts for this distribution?



Phenomenological selection model



Noisy but clear negative correlation between "chance to persist" and the typical growth rate

This motivated a simple evolution model, assuming constant problem and a rate of transition from one CTP state to another

$$\frac{\partial P}{\partial t} = -\alpha(x - \bar{x})P + \mu\frac{\partial^2 P}{\partial x^2}$$

Rate of change of distribution = net growth rate negatively correlated with x =CTP plus the effect of phenotypic changes modeled as unbiased random updates. Equilibrium solution is $P \sim Ai((\mu\alpha)^{1/3}(x - \overline{x})$

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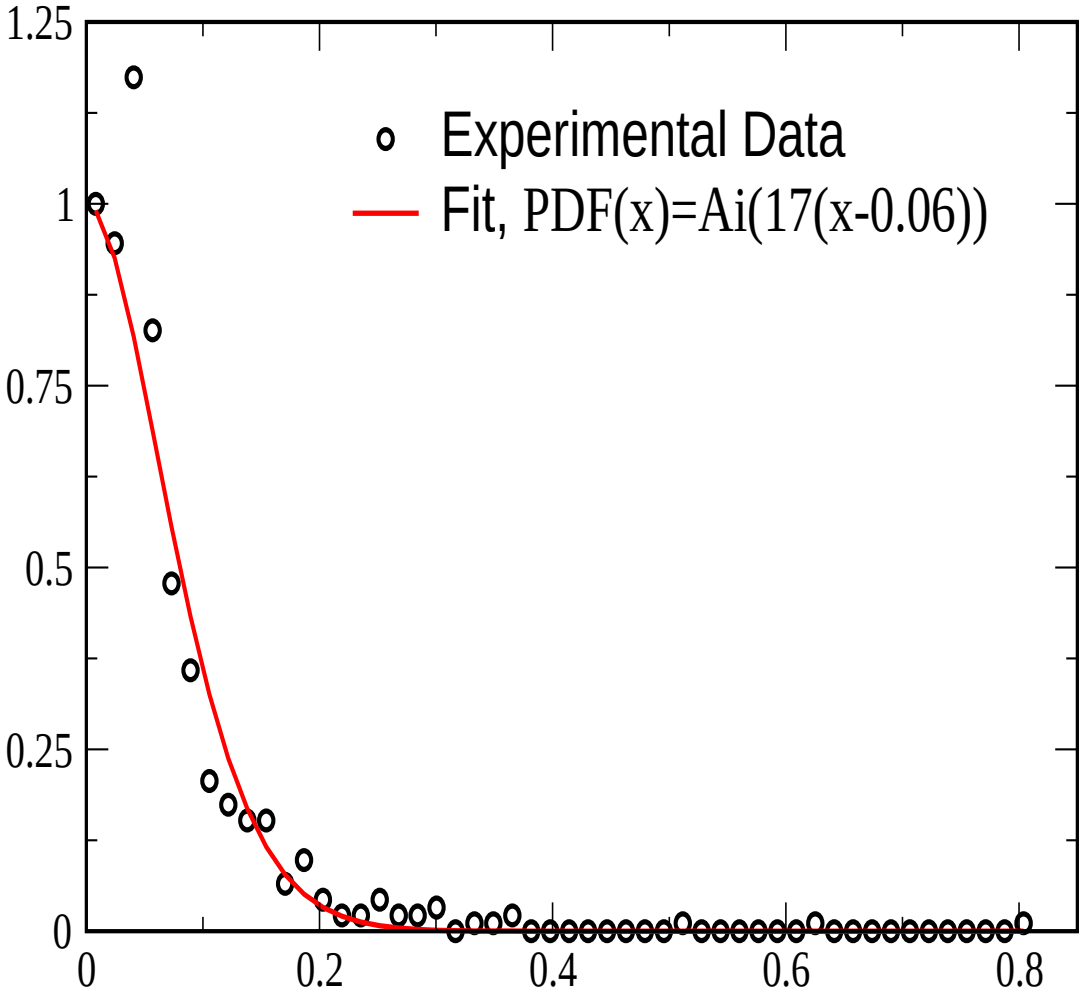
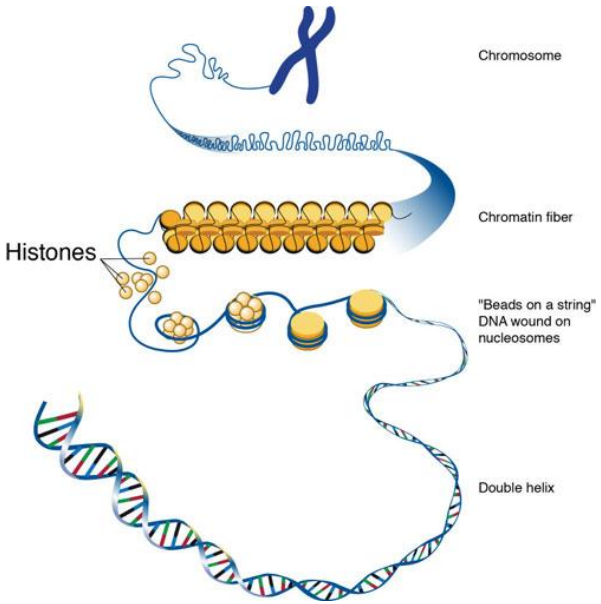
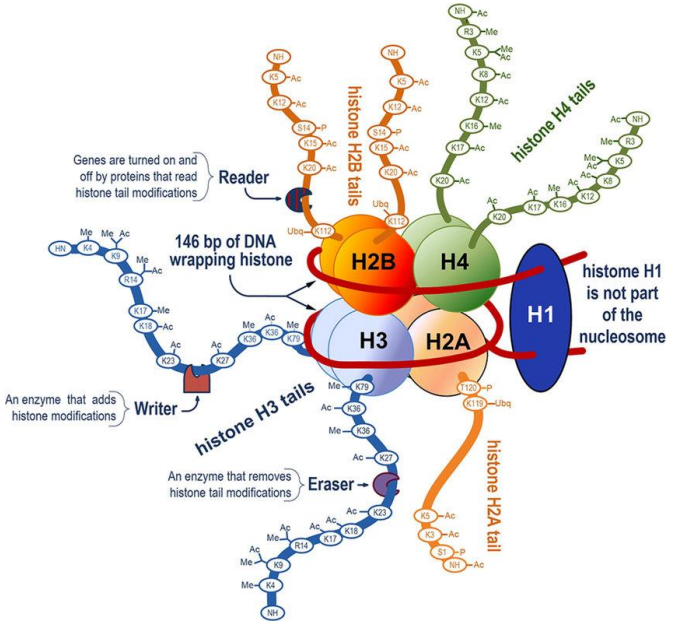


What is the biological cause of varying “x”?

- Answer undoubtedly has to do with epigenetic degrees of freedom





Post-translational modifications (both of DNA and histones) affect accessibility

From the literature

"A Chromatin-Mediated Reversible Drug-Tolerant State in Cancer Cell Subpopulations", Sharma, et al. Cell, 141, 69 (2010)

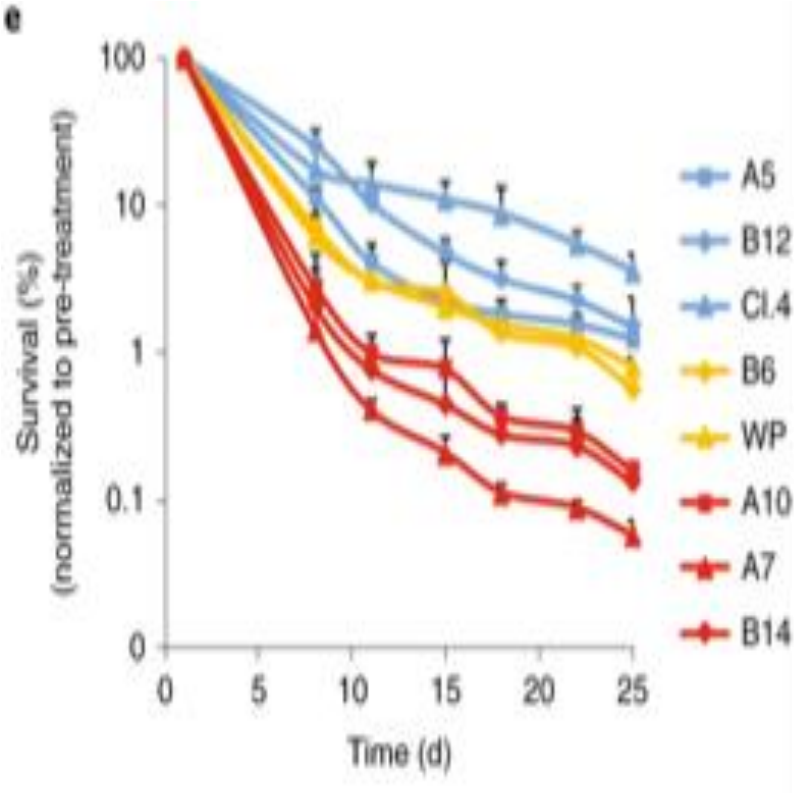
"Persister cells demonstrate >100-fold reduced drug sensitivity and maintain viability via engagement of IGF-1 receptor signaling and an altered chromatin state that requires the histone demethylase RBP2/KDM5A/Jarid1A"

Dumbrava, Mihai Gabriel, et al. "Single-cell resolution of an open chromatin signature in persister tumor cells." *Cell Reports* 45.1 (2026).
"Persister potential is encoded in a chromatin landscape. The findings reveal an intrinsic epigenetic program underlying chemotherapy tolerance."

Al-Radhawi, M. A., Tripathi, S., Zhang, Y., Sontag, E. D., & Levine, H. (2022). Epigenetic factor competition reshapes the EMT landscape. *Proceedings of the National Academy of Sciences*, *119*(42), e2210844119.
Epigenetic factors control the accessibility of cell states in other adaptation processes such as the epithelial-mesenchymal transition (EMT)

Note: Epigenetic marks are heritable for some time, but can eventually drift

Cells continue to die after Day 7



Persisting does not mean resisting; it means that the cell **individual phenotype** has found a means to counteract the effect of the drug. But this phenotype is not necessarily stable with respect to stochastic drift, which may bring it back to sensitivity

Again, we characterize this effect phenomenologically
Let **s** = survivability of a cell. **s** changes in time due to random diffusion, subject to constraint that mean survival after 7 days is given by x

$$\frac{\partial P_S(s,t)}{\partial t} = b\frac{\partial}{\partial s}\left((s-s_0)P_S\right) + \sigma^2\frac{\partial^2 P_S}{\partial s^2} - \theta(-s)kP_S$$

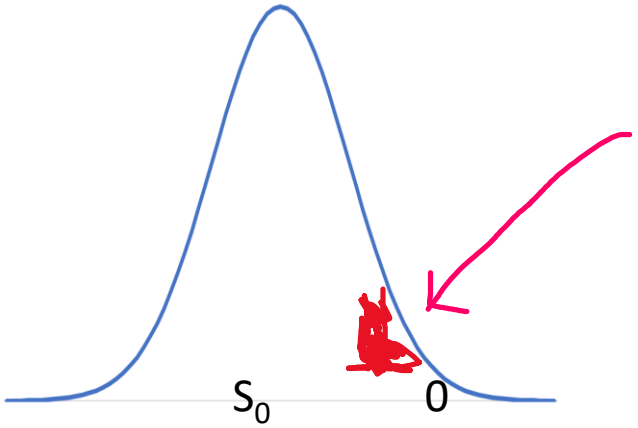
S = survivability

Deciphering the survivability distribution equation

$$\frac{\partial P_S(s,t)}{\partial t} = b\frac{\partial}{\partial s}\left((s-s_0)P_S\right) + \sigma^2\frac{\partial^2 P_S}{\partial s^2} - \theta(-s)kP_S$$

Time rate of change of the survivability curve for a specific clone is determined by

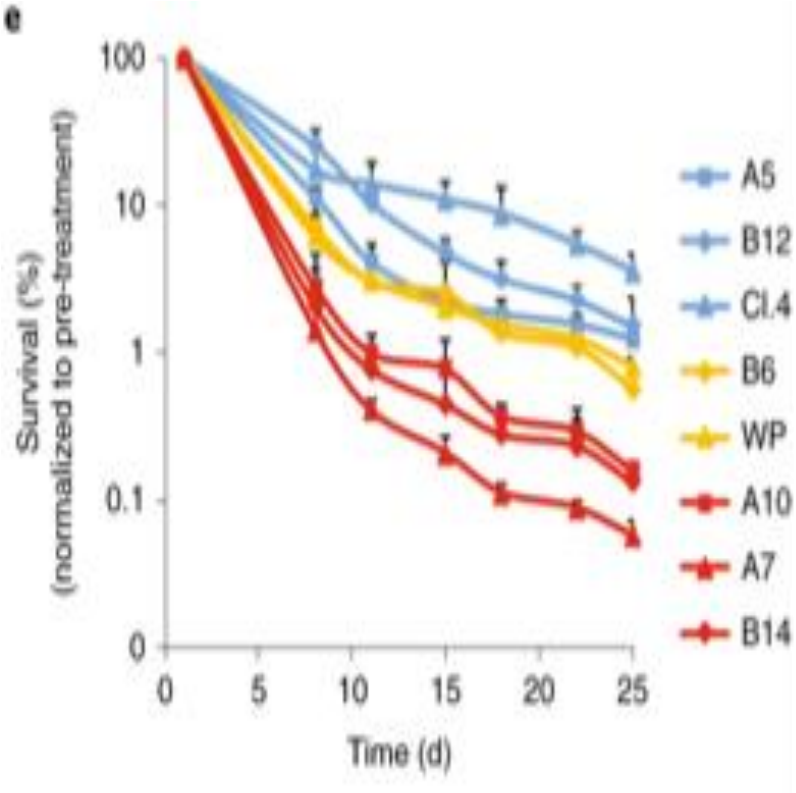
- Balance between random diffusion and the attraction to a mean value of survivability set by the chance to persist.
- The last term indicates that once the drug is administered, any cell with s < 0 will die very quickly, with rate k

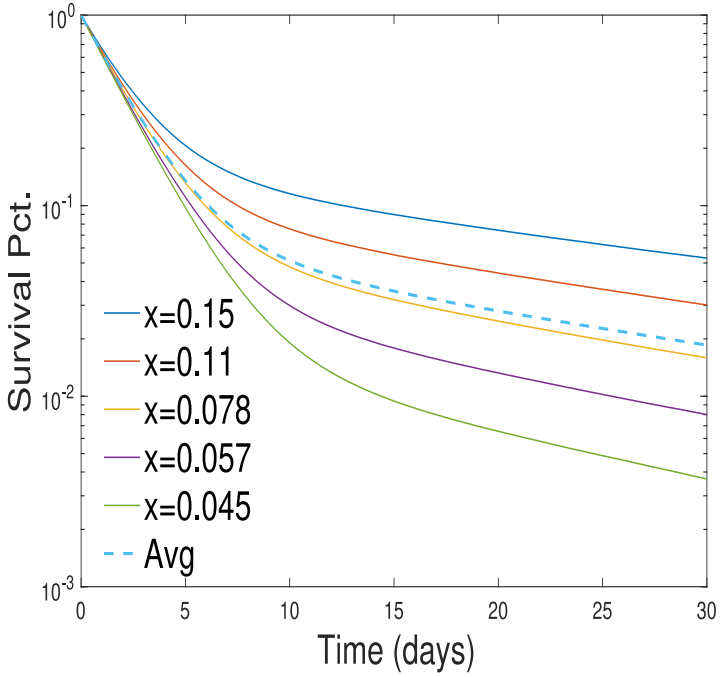


persisters

Note: we have ignored the possibility that drug stress leads to more rapid exploratory behavior; see e.g. work of Susan Rosenberg on bacteria

Cells continue to die after Day 7

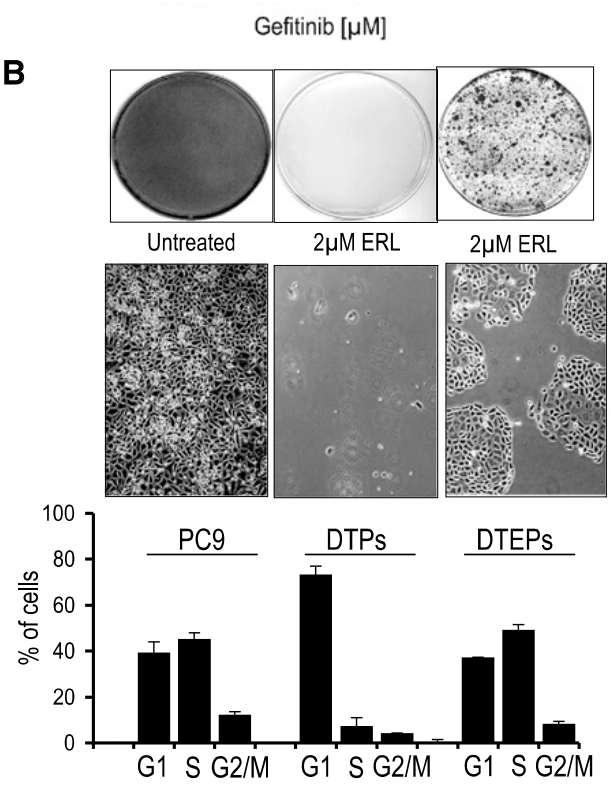




- Note that theory predicts higher persister percentage clones have slower decay rates
- Semi-quantitively consistent with experimental data

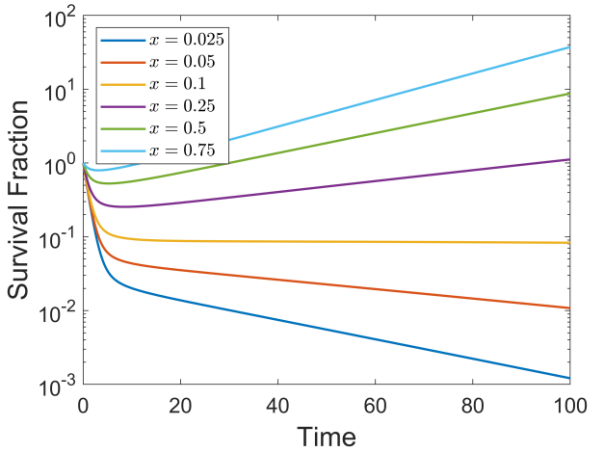
What is missing?

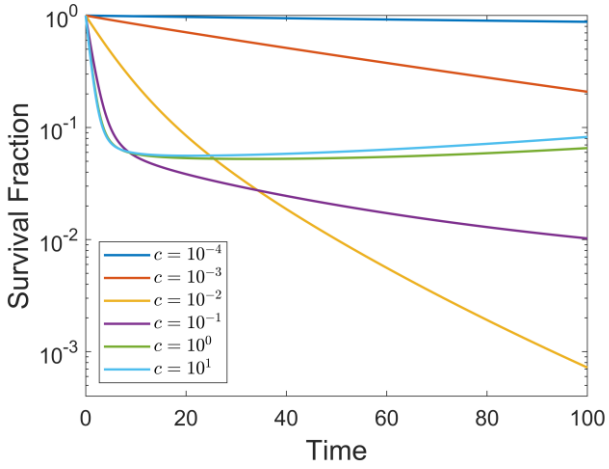
- Our baseline model does not account for the spontaneous transition to cycling persisters (DTEP's) – see Figure from Sharma et al (2010)
- Selection paradigm is not always sufficient; drug can directly induce more plasticity and even directional drift - see e.g. Russo,. Nat Genet 54, 976–984 (2022).
- So, we add an "Lamarckian" advection term to our PDE and also put in growth term at large **s**; see also H Chisholm, T Lorenzi, J Clairambault, Biochimica et Biophys. Acta - Gen. Subj. 1860, 2627–2645 (2016)



Extended equation

$$\frac{\partial P(x,s,t)}{\partial t} = \left(\mu \frac{\partial^2}{\partial x^2} + \sigma^2 \frac{\partial^2}{\partial s^2}\right) P + b \frac{\partial}{\partial s}(s - s_0(x) - v(s, c(t)))P) - k\big(s, c(t)\big)P.$$

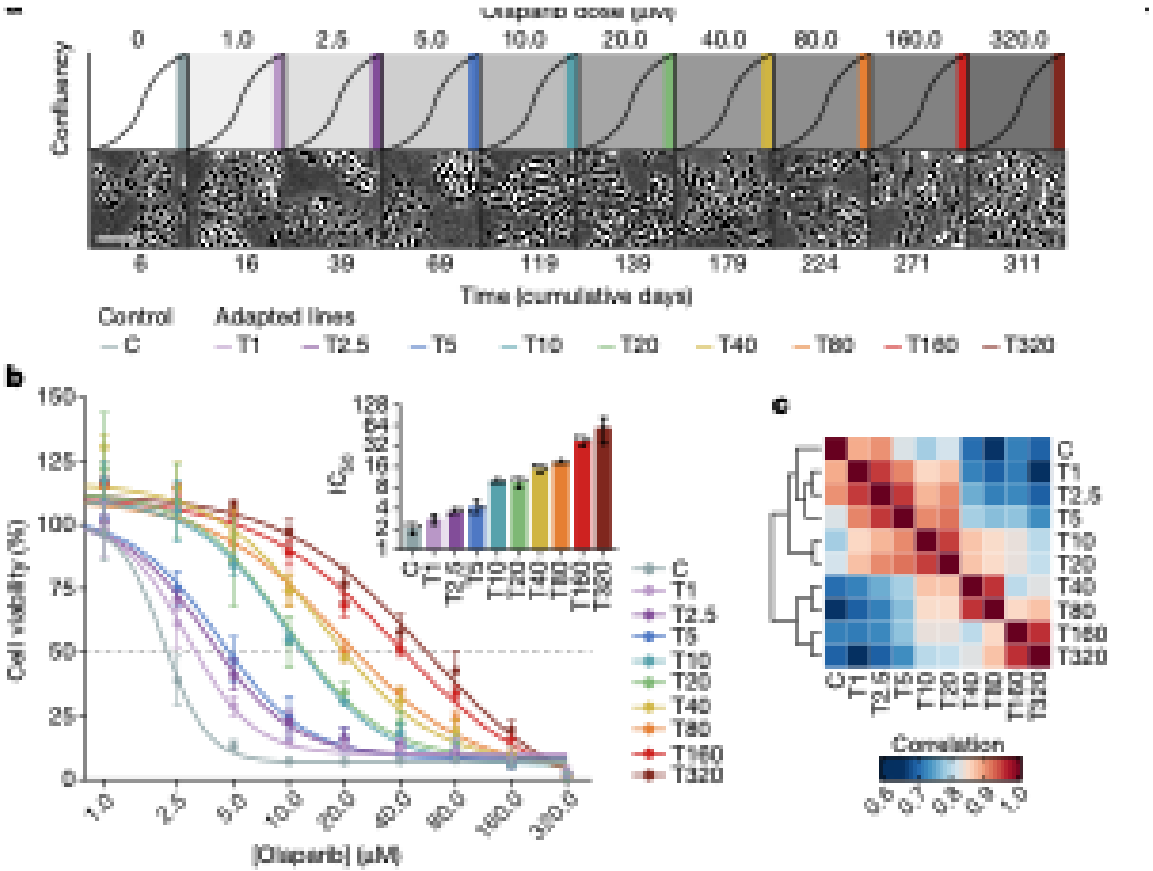




Transition to cycling persisters will occur if the population contains clones with high CTP; critical x can be analytically estimated

Dependence on drug level can be non-intuitive; low levels don't kill, high levels can cause better adaptation

A continuum of plasticity



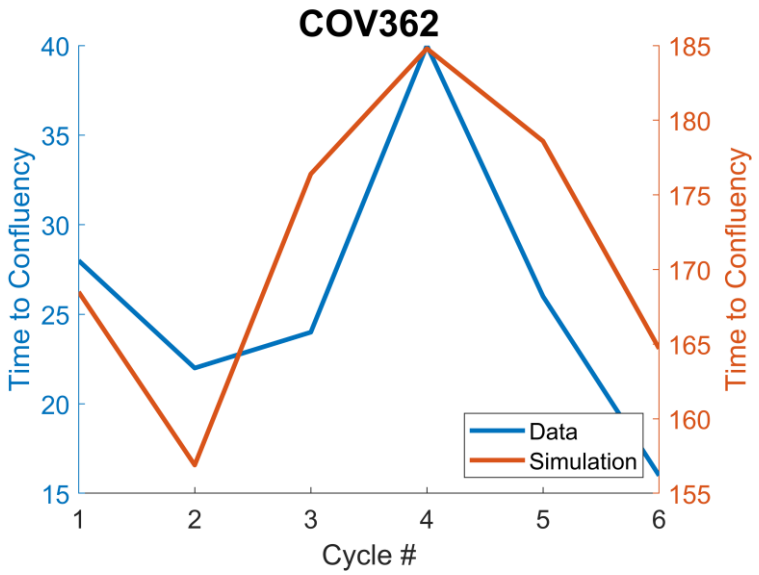
Add drug, wait until cycling persisters emerge and then grow until confluence

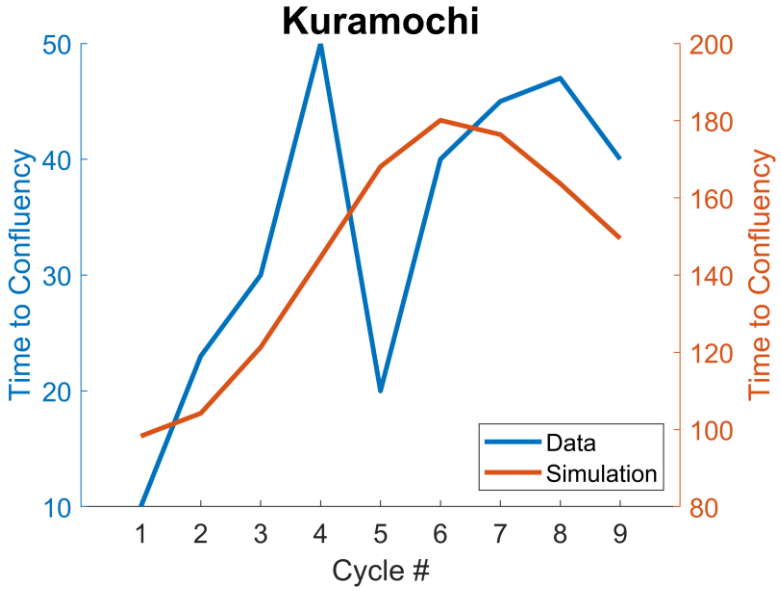
Increase drug dosage and repeat. Cells can be coaxed into much more effective persistence

França, Gustavo S., et al. "Cellular adaptation to cancer therapy along a resistance continuum." *Nature* 631.8022 (2024): 876-883.

Comparing to our model

Our continuous approach can directly compare to the macroscopic data in this study. Our preliminary results are encouraging (not perfect, but perhaps N=1 matters)





Many directions to investigate

- Global versus local plasticity
- The role of metabolism
- Relationship between persistence and dormancy
- Immune persisters

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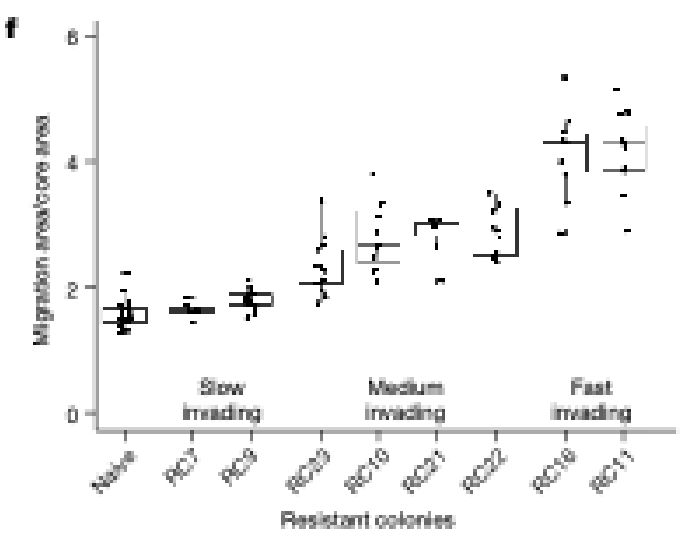
Different clones can take different paths

Resistant clones emerging from single-cell-derived cancer cells adopt molecularly, morphologically and functionally distinct resistant types. These resistant types are largely predetermined by molecular differences between cells before drug addition and not by extrinsic factors.



- As an example, different clones generated resistant strains with different degrees of invasiveness
- Clonal (epigenetic?) factors, determine the possible manifestations of cellular plasticity

Goyal, Yogesh, et al. "Diverse clonal fates emerge upon drug treatment of homogeneous cancer cells." *Nature* 620.7974 (2023): 651-659.

Many directions to investigate

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- The role of metabolism
- Relationship between persistence and dormancy
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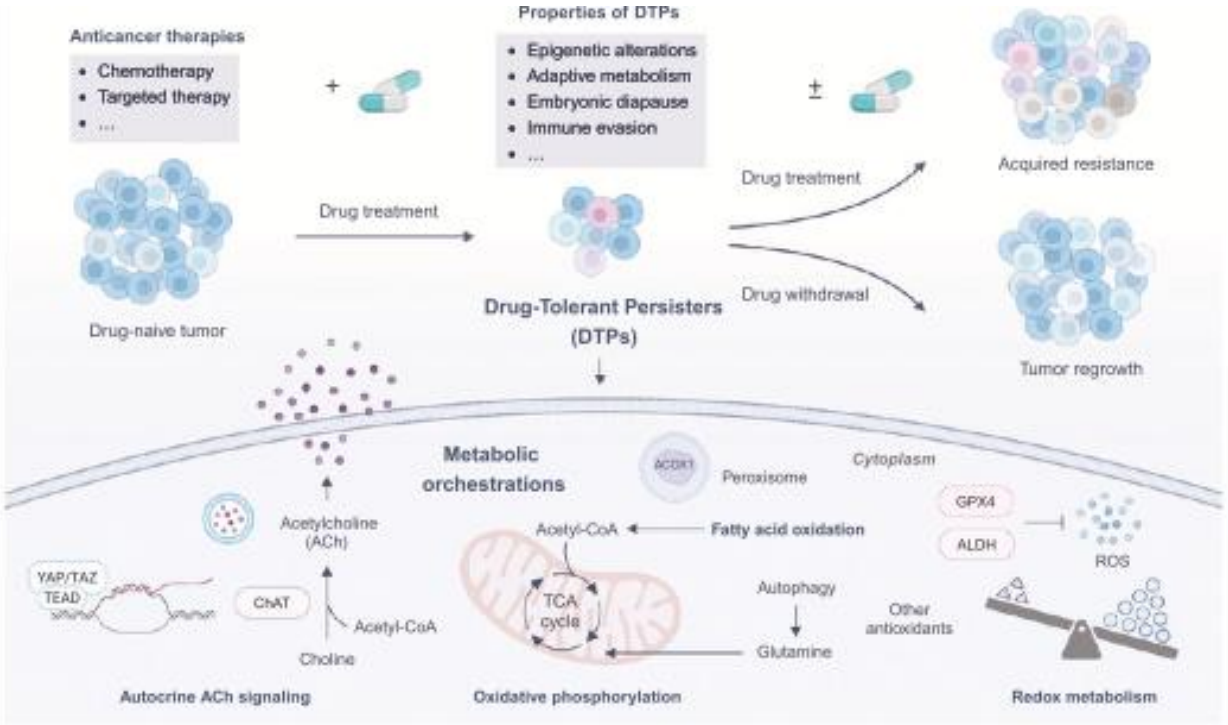
Metabolism plays a key role

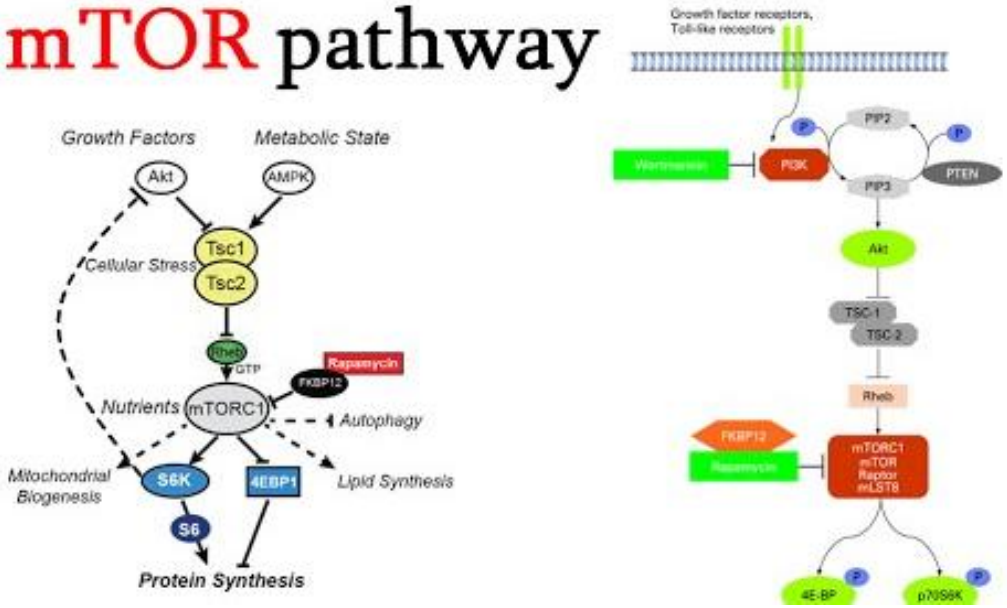


- Role of IGF, IRS1 in CTP
- Role of FAO in cycling
- Activation of NRF2
- Epigenetic connection?

Nie, Meng, and Zeping Hu. "Metabolic orchestration of drug-tolerant persister cells in cancer." *Life Medicine* 3.6 (2024).

Metabolism can provide a fitness signal enabling the increase in plasticity

- Suggestion: An integrated sensor of the metabolic state of the cell, such as provided by the mTOR pathway



Can couple directly tom chromatin via availability of metabolites needed for histone marking, e.g.

Connects to role of cell growth in global gene regulation, e.g. in E. Coli

Many directions to investigate

- Global versus local plasticity
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Drug Resistance	Dormancy
Stress due to oncogene inhibition	Stress due to foreign microenvironment
Some cells persist, exit cell cycle; in melanoma, some cell populations “idle”	Some cells go dormant and survive; also, populations can exhibit dormancy
Persisters can exhibit EMT/stemness markers	Dormant cells can exhibit EMT/stemness markers
Persisters can transition back to cycling, populations to net growth	Dormant cells can awaken; But seems to require an external stimulus
DTP, DTEP have distinct REDOX, metabolic properties; Cycling states use more OXPHOS and hence need NRF2, Glutathione	Dormant cell subtypes have distinct REDOX, metabolic properties; Awakening states use more OXPHOS and hence need NRF2, Glutathione
Eventually, full resistance can emerge	Eventually, full growth of differentiated cells can emerge

Many directions to investigate

- Global versus local plasticity
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Recent BioArxiv posting

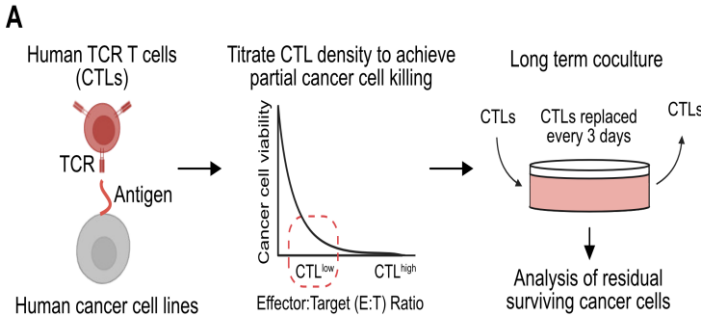
Drug tolerant persisters and immunotherapy persister cells exhibit cross-resistance and share common survival mechanisms

From lab of Anthony Letai, Dana-Farber Cancer Center

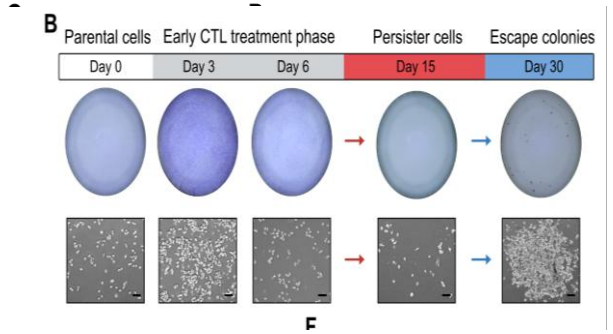
- Immunotherapy persister cells (IPCs) are less sensitive to drugs and radiation.
- Drug tolerant persisters (DTPs) are less sensitive to radiation and CAR T cell attack.
- IPCs and DTPs are less sensitive to mitochondrial apoptosis.
- Targeting anti-apoptotic dependencies helps eliminate IPCs/DTPs.

Some details

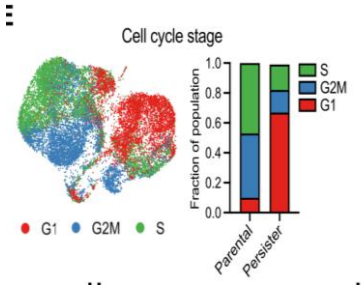
- In vitro co-culture with three melanoma cell lines; A375, SKMEL37 (both with NT-ESO-1 antigen) and MEL624 with MART1 antigen



- First two lines exhibit signs that they were effectively attacked by CTLs but avoided dying; MART1 cells dropped the antigen



- Mostly similar to expected behavior of persisters, namely cell cycle arrest in G1 and “neural-crest like signatures”



Summary

- Cancer cell plasticity is a critical component of many aspects of tumor progression
- We are studying a continuum model motivated the epigenetic control of phenotypic adaptation
- We can qualitatively (and sometime semi-quantitatively) explain various aspects of the persister phenomenon
- There are many more aspects of this class of behavior need to be considered as we move forward

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