The role of aneuploidy in the evolution of cancer drug resistance

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Abstract

Introduction

Aneuploidy in cancer. Chromosomal instability (CIN) is the mitotic process in which cells suffer from chromosome mis-segregation that leads to aneuploidy, where cells are characterized by structural changes of the chromosomes and copy number alterations (Schukken and Foijer, 2018). Interestingly, aberrations in chromosome copy number have been shown to allow cancer cells to survive under stressful conditions such as drug therapy. Indeed, cancer cells are often likely to be aneuploid, and aneuploidy is associated with poor patient outcomes (Ben-David and Amon, 2020).

The role of chromosomal instability (CIN) in the emergence of cancer has been studied extensively in the past decades (Christine et al., 2018, Komarova et al., 2003, Michor et al., 2005, Nowak et al., 2002, Pavelka et al., 2010, Zhu et al., 2018). One hypothesis is that CIN facilitates tumor genesis by accelerating the removal of tumor suppression genes (TSG) and subsequent appearance of cancer. The deletion of tumor suppression genes can happen in two ways: two point mutations deleting both alleles of the TSG (assuming a diploid genotype), or one point mutation and one chromosomal loss event. Initial theoretical studies have shown that aneuploidy can have a significant role in the deletion of the the tumor suppressing genes when compared to two consecutive point mutations (Komarova et al., 2008, 2003, Michor et al., 2005, Nowak et al., 2002). However, when taking into account that the appearance of aneuploidy requires a mutation to trigger CIN, the probability that CIN precedes tumor genesis is highly unlikely.

Evolutionary rescue. Populations adapted to a certain environment are vulnerable to environmental changes, which might cause extinction of the population. Examples of such environmental changes include climate change, invasive species or the onset of drug therapies. Adaptation is a race against time as the population size decreases in the new environment (Tanaka and Wahl, 2022). *Evolutionary rescue* is the process where the population acquires a trait that increases fitness in the new environment such that extinction is averted. It is mathematically equivalent to the problem of crossing of fitness valley (Weissman et al., 2009, 2010). There are three potential ways for a population to survive environmental change: migration to a new habitat similar to the one before the onset of environmental change (Cobbold and Stana, 2020); adaptation by phenotypic plasticity without genetic modification (Carja and Plotkin, 2017, 2019, Levien et al., 2021); and adaptation through genetic modifications, e.g., mutation (Uecker and Hermisson, 2011, 2016, Uecker et al., 2014).

Models of evolutionary rescue usually assume that the fitness of the wildtype and mutant are homogeneous in time. An exception was given by Marrec and Bitbol (2020), who modeled the fitness of the wildtype and mutant as time dependent. Additionally, Uecker and Hermisson (2011) investigated the probability of fixation of a beneficial mutation in a variable environment with arbitrary time-dependent selection coefficient and population size. Most models focus on the probability that at least one mutation rescues the population. How multiple mutations contribute to the survival of the population is less explored, but Wilson et al. (2017) have shown that evolutionary rescue is significantly enhanced by soft selective sweeps when multiple mutations contribute. Evolutionary rescue that requires two successive mutations has been investigated using diffusion approximation by Martin et al. (2013).

Methods

Evolutionary model

We follow the number of cancer cells that have one of three different genotypes at time t: wildtype, w_t; aneuploid, a_t; and mutant, m_t. These cells divide and die with rates λ_k and μ_k (for $k = w, a, m$). The difference between the division and death rate is $\Delta_k = \lambda_k - \mu_k$. We assume the population of cells is under a strong stress, such as drug therapy, to which the wildtype genotype is susceptible and

therefore $\Delta_w < 0$, whereas the mutant is resistant to the stress, $\Delta_m > 0$ We analyze three scenarios: in the first, aneuploid cells are partially resistant, $\Delta_m > \Delta_a > 0$; in the second, aneuploid cells are tolerant, $0 > \Delta_a > \Delta_w$ (see Brauner et al., 2016, for the distinction between susceptible, resistant, and tolerant); in the third, aneuploid cells are non-growing or "barely growing", that is, either slightly tolerant or slightly resistant, such that $\Delta_a \approx 0$. Wildtype cells may missegregate to become aneuploids at rate u. Both aneuploid and wildtype cells may mutate to become mutants at rate v, which we assume is lower than the division rates, $v < \min(\lambda_w, \lambda_a, \lambda_m)$. See Figure 1 for an illustration of the model.

Stochastic simulations

Simulations are performed using a *Gillespie algorithm* (Gillespie, 1976, 1977) implemented in Python (Van Rossum and Others, 2007). The simulation monitors the number of cells of each type: wildtype, aneuploid, and mutant. The wildtype population initially consists of w_0 cells, whereas the other cell types are initially absent.

The state of the stochastic system at time t is represented by the triplet (w_t, a_t, m_t). The following describes the events that may occur (right column), the rates at which they occur (middle column), and the effect these events have on the state (Figure 1):

$$
\begin{aligned}
(+1,0,0): &\quad \lambda_w w_t \quad \text{(birth of wildtype cell)},\\
(-1,0,0): &\quad \mu_w w_t \quad \text{(death of wildtype cell)},\\
(-1,+1,0): &\quad u w_t \quad \text{(wildtype cell becomes aneuploid)},\\
(-1,0,+1): &\quad v w_t \quad \text{(wildtype cell becomes mutant)},\\
(0,+1,0): &\quad \lambda_a a_t \quad \text{(birth of aneuploid cell)},\\
(0,-1,0): &\quad \mu_a a_t \quad \text{(death of aneuploid cell)},\\
(0,-1,+1): &\quad v a_t \quad \text{(aneuploid cell becomes mutant)},\\
(0,0,+1): &\quad \lambda_a m_t \quad \text{(birth of mutant cell)},\\
(0,0,-1): &\quad \mu_a m_t \quad \text{(death of mutant cell)}.
\end{aligned}
$$

Each iteration of the simulation loop starts with computing the rates ν_j of each event j. We then draw the time until the next event, Δt, from an exponential distribution whose rate parameter is the sum of the rates of all events, such that $\Delta t \sim Exp(\sum_j \nu_j)$. Then, we randomly determine which event occurred, where the probability for event j is $p_j = \nu_j / \sum_i \nu_i$. Finally, we update the number of cells of each type according to the event that occurred and update the time from t to $t + \Delta t$. We repeat these iterations until either the population becomes extinct (the number of cells of all types is zero) or the number of mutant cells is high enough so that its extinction probability is $< 0.1\%$, that is until

$$
m_t > \left\lfloor \frac{3 \log 10}{\log\left(\lambda_m / \mu_m\right)} \right\rfloor + 1.
$$

τ-leaping. When simulations are slow (e.g. due to large population size), we utilize τ-leaping (Gillespie, 2001), where change in number of cells of genotype i in a fixed time interval Δt is Poisson distributed with mean $\nu_i \Delta t$. If the change in number of cells is negative and larger then the subpopulation size then the subpopulation size is updated to be zero.

Density-dependent growth. In our analysis we assume that lineages produced by cells from the initial population divide and die independently of each other, which may be unrealistic, as cells usually compete for resources. A more realistic model includes competition for limited resources and spatial structure, which may play an important role in the development of cancer (e.g., Martens et al.,

2011). To simulate birth and death rates that depend on the number of cells in the population, we transform the rates of division and death to the following:

$$\begin{aligned}
\lambda'_w &= \lambda_w, \\
\mu'_w &= \mu_w, \\
\lambda'_a &= C_1 + (\lambda_a - \mu_a)\left(1 - \frac{w+a+m}{K}\right), \\
\mu'_a &= C_1, \\
\lambda'_m &= C_2 + (\lambda_m - \mu_m)\left(1 - \frac{w+a+m}{K}\right), \\
\mu'_m &= C_2,
\end{aligned}$$

where $C_1, C_2 > 0$ are constants and K is the maximum carrying capcity.

Code and data availability.

All source code is available online at https://github.com/yoavram-lab/EvolutionaryRescue.

Results

Evolutionary rescue probability

In our model, *evolutionary rescue* occurs when resistant cells appear and fixate ($m_t \gg 1$) in the population before the population becomes extinct ($w_t = a_t = m_t = 0$). Aneuploidy may contribute to evolutionary rescue by either preventing (when $\Delta_a > 0$) or delaying (when $0 > \Delta_a > \Delta_w$) the extinction of the population before mutant cells appear and fixate. We assume independence between clonal lineages starting from an initial population of N wildtype cells (we check the effect of density-dependent growth on our results below). We therefore define p_w as the probability that a lineage starting from a single wildtype cell avoids extinction by acquiring drug resistance. Thus, $N^* = 1/p_w$ is the threshold tumor size above which evolutionary rescue is very likely, and the rescue probability is given by

$$p_{\text{rescue}} = 1 - \left(1 - p_w\right)^N \approx 1 - \mathrm{e}^{-Np_w} = 1 - e^{-N/N^*}. \tag{2}$$

where the approximation $(1-p_w) \approx e^{-p_w}$ assumes that p_w (but not necessarily Np_w) is small. Indeed, when $N < 1/p_w$, then the probability for evolutionary rescue is $p_{\text{rescue}} \approx Np_w$ and when $N > 1/p_w$, it is $p_{\text{rescue}} \approx 1$, justifying the definition of N^* as the threshold tumor size.

In the Appendix, we use the theory of multi-type branching processes to find approximate expressions eqs. (9), (16) and (19) for p_w in different regimes. Substituting these into $N^* = 1/p_w$, we find approximations for the threshold tumor size, N^*. For these approximations, an important quantity is $T^* = (4v\lambda_a\Delta_m/\lambda_m)^{-1/2}$, which is the critical time an aneuploid lineage needs to survive to produce a resistant mutant that avoids random extinction. First, if aneuploidy is very rare ($uT^* < 1$), or if aneuploidy is rare ($u < -\Delta_a$) and very sensitive to the drug ($\Delta_a T^* < -1$), then rescue will likely occur by a direct resistance mutation in a sensitive cell, such that

$$N^*_m \approx \frac{|\Delta_w|}{v} \cdot \frac{\lambda_m}{\Delta_m}. \tag{3}$$

Here, $|\Delta_w| / v$ is the ratio of the rate at which wild-type cells are decreasing in number and the rate at which they are mutating.

Otherwise, aneuploidy is frequent enough ($u > \max(-\Delta_a, 1/T^*)$) to affect the evolution of drug resistance. The threshold tumor size, N^*, can then be approximated by one of the following cases, depending on $\Delta_a T^*$, the change in the log of the aneuploid population size during the critical time,

$$N_a^* \approx \frac{|\Delta_w|}{u} \cdot \begin{cases} \frac{|\Delta_a|}{v} \cdot \frac{\lambda_m}{\Delta_m}, & \Delta_a T^* \ll -1 \text{ (partially sensitive aneuploids)}, \\ 2\lambda_a T^*, & -1 \ll \Delta_a T^* \ll 1 \text{ (stationary aneuploids)}, \\ \frac{\lambda_a}{\Delta_a}, & \Delta_a T^* \gg 1 \text{ (resistant aneuploids)}. \end{cases} \tag{4}$$

The first line describes the case in which aneuploids are still effectively killed by the treatment, but not as quickly as the wild type. In the second case, the aneuploids are sufficiently resistant that the size of each aneuploid lineage is expected to remain roughly constant. In both of these first two cases, aneuploidy increases the probability of rescue by slowing or halting the decrease of the cancer population, allowing more opportunities for producing resistant mutants. In the third case, aneuploidy provides sufficient resistance for the aneuploid population to re-grow the tumor even without additional resistance mutations. Note that in this case there is no dependence on the parameters characterizing mutants or their production (v, λ_m, and Δ_m). Comparing these approximations to results of stochastic evolutionary simulations, we find that the approximations perform very well (**????????**).

Using eqs. (3) and (4), we can find the ratio of threshold tumor size for rescue via aneuploidy (u is high) or via direct mutation (u is low),

$$\frac{N_a^*}{N_m^*} \approx \begin{cases} \frac{|\Delta_a|}{u}, & \Delta_a T^* \ll -1, \\ \frac{1}{u}\left(\frac{\lambda_a}{v} \cdot \frac{\lambda_m}{\Delta_m}\right)^{1/2}, & -1 \ll \Delta_a T^* \ll 1, \\ v\frac{\Delta_m}{\lambda_m} \cdot \left(u\frac{\Delta_a}{\lambda_a}\right)^{-1}, & \Delta_a T^* \gg 1. \end{cases} \tag{5}$$

In all cases, the effect of aneuploidy increases (i.e., the threshold size ratio decreases) when the aneuploidy rate u increases. Increasing the aneuploid growth rate Δ_a also leads to an increased role of aneuploidy, although the effect is minor when $|\Delta_a|$ is small compared to T^*.

In the first case, $|\Delta_a| / u$ is the ratio of the expected time for an aneuploid lineage to appear, $1/u$, and the expected time until that lineage disappears, $1/\Delta_a$. In the third case, $\left(v\frac{\Delta_m}{\lambda_m}\right) / \left(u\frac{\Delta_a}{\lambda_a}\right)$ is the ratio of the rates of formation of resistant mutants that avoid extinction and partially resistant aneuploids that avoid extinction. In the second case, $\frac{1}{u}\left(\frac{\lambda_a}{v} \cdot \frac{\lambda_m}{\Delta_m}\right)^{1/2} = \sqrt{\frac{\Delta_a}{u} \cdot v\frac{\Delta_m}{\lambda_m} \cdot \left(u\frac{\Delta_a}{\lambda_a}\right)^{-1}}$, which is the geometric mean of the first and third cases.

Interestingly, increasing both the aneuploid division rate, λ_a, and the aneuploid death rate, μ_a, such that the growth rate Δ_a remains constant, leads to decreases in T^*, and therefore to the second case. In this case, increasing the division rate λ_a should also increase the mutation rate v in aneuploid cells, as mutations mostly occur during division , so overall the threshold tumor size N_a^* is unaffected by the division rate λ_a (i.e., $d\lambda_a T^*/d\lambda_a = 0$). Thus, if aneuploids rapidly die due to the drug but compensate by rapidly dividing, further increasing the division rate will *not* facilitate adaptation.

Density-dependent growth. In our analysis we used branching processes, which assume that growth (division and death) is density-independent. However, growth may be limited by resources (oxygen, nutrients, etc.) and therefore depend on cell density. We therefore performed stochastic simulations of a logistic growth model with carrying capacity K (see Methods). We find that our approximations agree with results of simulations with density-dependent growth for biologically relevant parameter values (**??**).

Standing vs. de-novo genetic variation. In the above we assumed that at the onset of drug treatment, the initial tumor consisted entirely of wildtype cells that are drug sensitive. However, aneuploid cells are likely generated even before onset of treatment at some rate $\tilde{u} \leq u$ (because the treatment itself may promote generation of aneuploid cells REF), which are likely to have a deleterious effect in the absence of the drug, s (REF). But if the number of cells in the tumor N is large (as expected if the tumor is to be treated with a drug), there may already be a fraction $f \approx \tilde{u}/s$ of aneuploid cells in the population.

Therefore, the threshold tumor size with standing generation variation, $\tilde{N}_a^*$, is similar to the ratio with de-novo variation, N_a^*, except that the sensitive growth rate $|\Delta_w|$ is replaced with the aneuploidy cost, s, such that

$$\frac{\tilde{N}_a^*}{N_a^*} = \frac{u}{\tilde{u}} \frac{s}{|\Delta_w|}. \tag{6}$$

Therefore, standing genetic variation will drive adaptation to the drug if Δ_w is very negative due to a stronger effect of the drug on sensitive cells, or if s is very small due to a low cost of aneuploidy in the pre-drug conditions. In contrast, de-novo aneuploids will have a stronger effect on adaptation if the aneuploidy cost s is large, the effect of the drug is weak (Δ_w is small), or if the drug induces the appearance of aneuploid cells ($u > \tilde{u}$).

Recurrence time due to evolutionary rescue

Even when evolutionary rescue occurs and leads to recurrence of the tumor, it may take a long time. The overall expected recurrence time can be estimated by adding two terms: the mean waiting time for evolutionary rescue–the appearance of a resistant lineage that avoid extinction–and the expected time for proliferation of that lineage back to the original tumor size, N.

Evolutionary rescue time. In Appendix C we derive an approximation for τ_1, the expected rescue time without aneuploidy ($u = 0$), and τ_2, the expected rescue time with aneuploidy ($u > 0$). **??** shows the agreement between these approximations and simulation results for intermediate and large tumor sizes.

Proliferation time. TODO

Discussion

We have modeled a tumor–a population of cancer cells–exposed to drug treatment that causes the population to decline in size towards potential extinction. In this scenario, the tumor can be "evolutionary rescued", or escape extinction, via two paths. In the direct path, a sensitive cell acquires a mutation that confers resistance that allows it to rapidly grow. In the indirect path, a sensitive cell first becomes aneuploid, which diminishes the effect of the drug, and then an aneuploid cell acquires a mutation that confers resistance (Figure 1).

Using multitype branching processes, we derived the probability of evolutionary rescue of the population of cancer cells under different scenarios for the effect of aneuploidy, ranging from tolerance to partial resistance. We obtained exact and approximate expressions for the probability of evolutionary rescue (eq. (2)). Our results show that the probability of evolutionary rescue increases with the initial tumor size N, the sensitive growth rate Δ_w, the mutation rate v, and the aneuploidy rate u.

When aneuploid cells are partially resistant to the drug ($\Delta_w \ll 0 \ll \Delta_a \ll \Delta_m$), evolutionary rescue can be approximated by a one-step process in which aneuploidy itself rescues the population (**??**). When aneuploidy only provides tolerance to the drug ($\Delta_w \ll \Delta_a \ll 0 \ll \Delta_m$), it cannot rescue the population. Instead, it acts as a *stepping stone* through which the resistant mutant can appear more

rapidly, given that the aneuploid cell population declines slower then the sensitive cell population. In this case, aneuploidy provides two benefits. First, it delays the extinction of the population–providing more time for appearance of the resistance mutation. Second, it increases the population size relative to a sensitive population–providing more cells in which mutations can occur, i.e., it increases the mutation supply, Nv.

We find that aneuploidy can have a significant effect on evolutionary rescue (**????**). For example, when aneuploidy cells are "barely-resistant" (they grow at a very low rate, $\Delta_a = 10^{-3}$) the probability of evolutionary rescue is 1,000-fold higher with aneuploidy than without it (for parameters previously described in cancer, see Table 1). Interestingly, aneuploidy is unlikely to contribute to evolutionary rescue in primary tumors in which the number of cells is large enough ($N > 10^7$) for the appearance of resistant mutation directly in sensitive cells before these cells become extinct (**??**). However, aneuploidy can have a crucial role in evolutionary rescue of secondary tumors, in which the number of sensitive cells may be below the detection threshold of $\sim 10^7$ (Bozic et al., 2013). Given the fact that the mean time for such secondary tumors to overcome chemotherapy can be of the order of 100 days (**??**), this can explain the reappearance of cancer even after initial remission. Indeed, we find that the tumor size can decrease by orders of magnitude before it is rescued (**??**).

We hypothesized that presence of *standing variation* –the existence of a subpopulation of aneuploid cancer cells before therapy begins–can facilitate evolutionary rescue by reducing the waiting time for the appearance of aneuploid cells. Indeed, we observe that even when a small fraction of the initial tumor is aneuploid, evolutionary rescue is more likely to occur through this existing standing variation, rather then through *de novo* aneuploid cells (**??**).

We have assumed that cancer cell lineages are independent of each other. However, this may not be the case, as cancer cells compete for resources (e.g., blood supply). Nevertheless, we find that when the carrying capacity if large our approximation for the probability of evolutionary rescue agrees with results of stochastic simulations with density-dependent growth **??**). Future work may focus on scenarios with small carrying capacity by analyzing density-dependent branching processes.

Our model predictions may be tested by experiments (Martin et al., 2013). For example, to study the effects of initial tumor size on the probability of evolutionary rescue, a large culture mass can be propagated from a single cancer cell in permissive conditions and then diluted to a range of starting tumor sizes. Afterwards, these tumors may be exposed to anti-cancer drugs that induces aneuploidy or to saline solution for control. Cell density can then be measured and compared to the predictions of our model.

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	Name	Value	Units	References
N	Initial tumor size	$10^7 - 10^9$	cells	Del Monte (2009)
λ_w	Wildtype division rate	0.14	1/days	Bozic et al. (2013)
μ_w	Wildtype death rate	0.17	1/days	Bozic et al. (2013)
λ_a	Aneuploid division rate*	0.14	1/days	-
μ_a	Aneuploid death rate*	0.13 – 0.17	1/days	-
λ_m	Mutant division rate	0.14	1/days	Bozic et al. (2013)
μ_m	Mutant death rate	0.13	1/days	Bozic et al. (2013)
u	Missegregation rate	$10^{-3} - 10^{-2}$	1/cell division	Bakker et al. (2023), Nowak et al. (2004)
v	Mutation rate	$10^{-7} - 10^{-9}$	1/gene/cell division	Nowak et al. (2004)

Table 1: Model parameters. NEED DIFFERENT REFS—THESE ARE MOSTLY THEORY PAPERS. CHECK BIONUMBERS? Aneuploid birth rate λ_a is set to the same value as the wildtype and mutant birth rates, λ_w and λ_m. Aneuploid death rate μ_a is set to an intermediate value between the wildtype and mutant death rates, μ_w and μ_m.

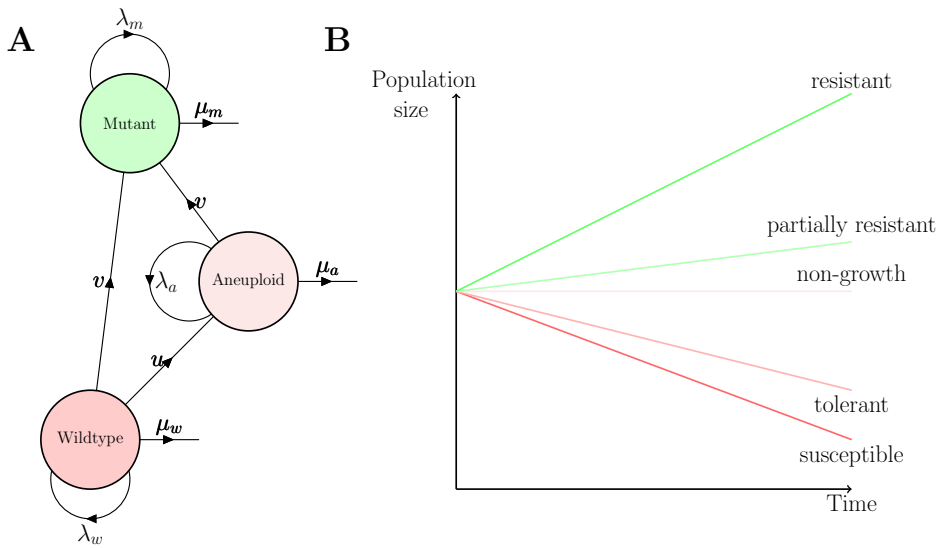

Figure 1: Model illustration. **(A)** A population of cancer cells is composed of wildtype, aneuploid, and mutant cells, which divide with rates λ_w, λ_a, and λ_m and die at rates μ_w, μ_a, and μ_m, respectively. Wildtype cells can become aneuploid at rate u. Both aneuploid and wildtype cells can acquire a beneficial mutation with rate v. Color denotes the relative growth rates of the three genotypes such that $\lambda_w - \mu_w < \lambda_a - \mu_a < \lambda_m - \mu_m$. **(B)** The wildtype and the mutant are susceptible and resistant, respectively, to the drug. The aneuploid may be tolerant, non-growing, or or partially resistant.

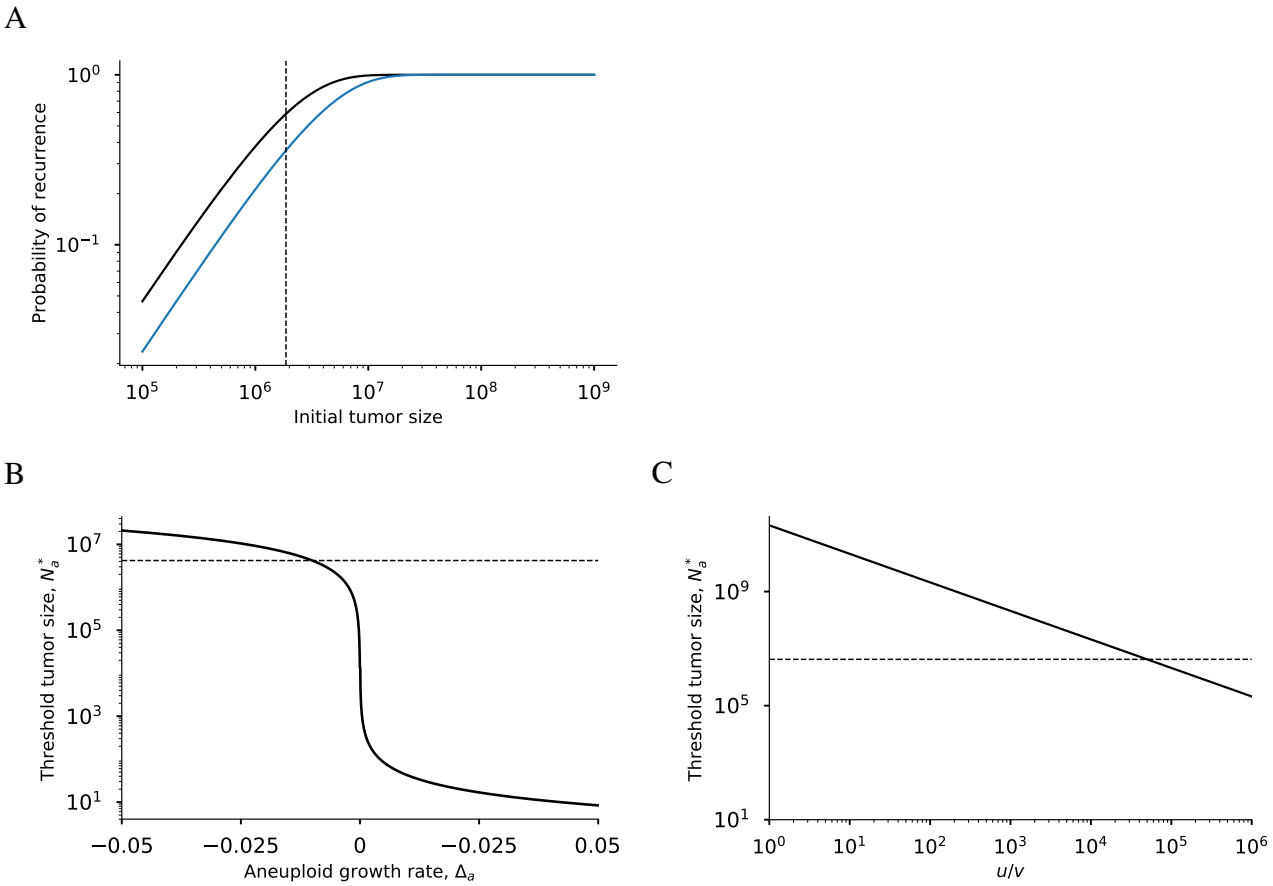

Figure 2: Aneuploidy facilitates evolutionary rescue of cancer under drug treatment. **(A)** The probability of evolutionary rescue (i.e. the probability that the population does not go to extinction), p_{rescue}, as a function of the initial tumor size, N. Dashed line shows the threshold tumor size, N^*, above which the probability is very high. **(B)** The threshold tumor size N_a^* as a function of the aneuploid growth rate Δ_a. The dashed horizontal line shows N_m^*, the threshold tumor size without aneuploidy ($u = 0$). When aneuploid growth rate is close to or higher than zero, aneuploidy decreases the threshold tumor size, thereby facilitating evolutionary rescue. **(C)** The threshold tumor size N_a^* as a function of the ratio of aneuploidy and mutation rates, u/v. The dashed horizontal line shows N_m^*, the threshold tumor size without aneuploidy ($u = 0$). When the aneuploidy rate is much higher than the mutation rate, aneuploidy decreases the threshold tumor size, thereby facilitating evolutionary rescue.

Appendices

Appendix A: Survival probability of a single lineage

To analyze evolutionary rescue in this model, we use the framework of *multitype branching processes* (Harris et al., 1963, Weissman et al., 2009). This allows us to find explicit expressions for the *survival probability*: the probability that a lineage descended from a single cell does not become extinct.

Let p_w, p_a, and p_m be the survival probabilities of a population consisting initially of single wildtype cell, aneuploid cell, or mutant cell, respectively. The complements $1-p_w$, $1-p_a$, and $1-p_m$ are the extinction probabilities, which satisfy each its respective equation,

$$\begin{aligned} 1-p_w =& \frac{\mu_w}{\lambda_w+\mu_w+u+v} + \frac{u}{\lambda_w+\mu_w+u+v}\left(1-p_a\right) + \\ & \frac{\lambda_w}{\lambda_w+\mu_w+u+v}\left(1-p_w\right)^2 + \frac{v}{\lambda_w+\mu_w+u+v}\left(1-p_m\right), \\ 1-p_a =& \frac{\mu_a}{\lambda_a+\mu_a+v} + \frac{v}{\lambda_a+\mu_a+v}\left(1-p_m\right) + \frac{\lambda_a}{\lambda_a+\mu_a+v}\left(1-p_a\right)^2, \\ 1-p_m =& \frac{\mu_m}{\lambda_m+\mu_m} + \frac{\lambda_m}{\lambda_m+\mu_m}\left(1-p_m\right)^2. \end{aligned} \tag{7}$$

The survival probabilities are given by the smallest solution for each quadratic equation (Uecker et al., 2015). Therefore we have

$$\begin{aligned} p_w &= \frac{\lambda_w-\mu_w-u-v+\sqrt{\left(\lambda_w-\mu_w-u-v\right)^2+4\lambda_w\left(up_a+vp_m\right)}}{2\lambda_w}, \\ p_a &= \frac{\lambda_a-\mu_a-v+\sqrt{\left(\lambda_a-\mu_a-v\right)^2+4\lambda_a v p_m}}{2\lambda_a}, \\ p_m &= \frac{\lambda_m-\mu_m}{\lambda_m}. \end{aligned} \tag{8}$$

Note that the equation for p_w depends on both p_a and p_m, and the equation for p_a depends on p_m. To proceed, we can plug the solution for p_m and p_a into the solution for p_w. We perform this for three different scenarios.

Scenario 1: Aneuploid cells are partially resistant

We first assume that aneuploidy provides partial resistance to drug therapy, $\lambda_a > \mu_a$, and that this resistance is significant, $\left(\lambda_a-\mu_a-v\right)^2 > 4\lambda_a v p_m$. We thus rewrite eq. (8) as

$$\begin{aligned} p_w &= \frac{\lambda_w-\mu_w-u-v}{2\lambda_w}\left(1-\sqrt{1+\frac{4\lambda_w\left(vp_m+up_a\right)}{\left(\lambda_w-\mu_w-u-v\right)^2}}\right), \text{and} \\ p_a &= \frac{\lambda_a-\mu_a-v}{2\lambda_a}\left(1+\sqrt{1+\frac{4\lambda_a v p_m}{\left(\lambda_a-\mu_a-v\right)^2}}\right). \end{aligned}$$

Using the quadratic Taylor expansion $\sqrt{1+x} = 1+x/2+O(x^2)$ and assuming $u, v \ll 1$, we obtain the following approximation for the survival probability of a population initially consisting of a single

wildtype cell,

$$p_w \approx -\frac{vp_m + up_a}{\lambda_w - \mu_w - u - v} \tag{9}$$

$$\approx -\frac{1}{\lambda_w - \mu_w}\left[\frac{u\left(\lambda_a - \mu_a\right)}{\lambda_a} + \frac{uv\left(\lambda_m - \mu_m\right)}{\lambda_m\left(\lambda_a - \mu_a\right)} + \frac{v\left(\lambda_m - \mu_m\right)}{\lambda_m}\right] \tag{10}$$

Now uv is very small, and if we assume $v \ll u$, we have

$$p_w \approx \frac{u}{|\Delta_w|} \cdot \frac{\Delta_a}{\lambda_a}. \tag{11}$$

Second-order approximation. To improve our approximation, we can consider the second term of the Taylor series expansion,

$$\left(1 + \frac{4\lambda_a v p_m}{\left(\lambda_a - \mu_a - v\right)^2}\right)^{\frac{1}{2}} = 1 + \frac{2\lambda_a v p_m}{\left(\lambda_a - \mu_a - v\right)^2} - \frac{\left(\lambda_a v p_m\right)^2}{4\left(\lambda_a - \mu_a - v\right)^4} + \cdots,$$

which gives us the following approximation,

$$p_a \approx \frac{\lambda_a - \mu_a - v}{\lambda_a} + \frac{vp_m}{\lambda_a - \mu_a - v} - \frac{\lambda_a\left(vp_m\right)^2}{8\left(\lambda_a - \mu_a - v\right)^3}. \tag{12}$$

We therefore have

$$p_w \approx -\frac{1}{\lambda_w - \mu_w - u - v}\left[\frac{u\left(\lambda_a - \mu_a - v\right)}{\lambda_a} + \frac{uv\left(\lambda_m - \mu_m\right)}{\lambda_m\left(\lambda_a - \mu_a - v\right)} + \frac{v\left(\lambda_m - \mu_m\right)}{\lambda_m} - \frac{uv^2\lambda_a\left(\lambda_m - \mu_m\right)^2}{8\lambda_m^2\left(\lambda_a - \mu_a - v\right)^3}\right]$$

$$\approx -\frac{1}{\lambda_w - \mu_w}\left[\frac{u\left(\lambda_a - \mu_a\right)}{\lambda_a} + \frac{uv\left(\lambda_m - \mu_m\right)}{\lambda_m\left(\lambda_a - \mu_a\right)} + \frac{v\left(\lambda_m - \mu_m\right)}{\lambda_m} - \frac{uv^2\lambda_a\left(\lambda_m - \mu_m\right)^2}{8\lambda_m^2\left(\lambda_a - \mu_a\right)^3}\right], \tag{13}$$

and using $\Delta_k = \lambda_k - \mu_k$, we can write the above equation as

$$p_w \approx -\frac{1}{\Delta_w}\left(\frac{u\Delta_a}{\lambda_a} + \frac{uv\Delta_m}{\lambda_m\Delta_a} + \frac{v\Delta_m}{\lambda_m} - \frac{uv^2\lambda_a\Delta_m^2}{8\lambda_m^2\Delta_a^3}\right). \tag{14}$$

Scenario 2: Aneuploid cells are tolerant.

We now assume that aneuploidy provides tolerance to drug therapy, that is, the number of aneuploid cells significantly declines over time, but at a lower rate than the number of wildtype cells, $\lambda_w - \mu_w < \lambda_a - \mu_a < 0$. We also assume that the decline are significant, $\left(\lambda_a - \mu_a - v\right)^2 > 4\lambda_a v p_m$. We rewrite eq. (8) as

$$p_w = \frac{\lambda_w - \mu_w - u - v}{2\lambda_w}\left(1 - \sqrt{1 + \frac{4\lambda_w\left(vp_m + up_a\right)}{\left(\lambda_w - \mu_w - u - v\right)^2}}\right), \text{and}$$

$$p_a = \frac{\lambda_a - \mu_a - v}{2\lambda_a}\left(1 - \sqrt{1 + \frac{4\lambda_a v p_m}{\left(\lambda_a - \mu_a - v\right)^2}}\right). \tag{15}$$

Since $u, v \ll 1$, the term in the root can be approximated using a 1st-order Taylor expansion. So, substituting the expressions for p_a and p_m, we have

$$\begin{aligned} p_w &\approx -\frac{vp_m + up_a}{\lambda_w - \mu_w - u - v} \\ &\approx \frac{1}{\lambda_w - \mu_w - u - v}\left[\frac{uv\left(\lambda_m - \mu_m\right)}{\lambda_m\left(\lambda_a - \mu_a - v\right)} - \frac{v\left(\lambda_m - \mu_m\right)}{\lambda_m}\right] \\ &\approx \frac{v\left(\lambda_m - \mu_m\right)}{\lambda_m\left(\lambda_w - \mu_w\right)}\left[\frac{u}{\left(\lambda_a - \mu_a\right)} - 1\right] \\ &= \frac{v\Delta_m}{\lambda_m|\Delta_w|}\left(\frac{u}{|\Delta_a|} + 1\right). \end{aligned} \tag{16}$$

Scenario 3: Aneuploid cells are non-growing

We now assume that the growth rate of aneuploid cells is close to zero (either positive or negative), such that $\left(\lambda_a - \mu_a - v\right)^2 < 4\lambda_a v p_m$. We rewrite eq. (8) as

$$p_a = \frac{\lambda_a - \mu_a - v + 2\sqrt{\lambda_a v p_m}\left(1 + \frac{\left(\lambda_a - \mu_a - v\right)^2}{4\lambda_a v p_m}\right)^{\frac{1}{2}}}{2\lambda_a}. \tag{17}$$

Using a following Taylor series expansion for small $\left(\lambda_a - \mu_a - v\right)^2 / 4\lambda_a v p_m$,

$$\left(1 + \frac{\left(\lambda_a - \mu_a - v\right)^2}{4\lambda_a v p_m}\right)^{\frac{1}{2}} = 1 + \frac{\left(\lambda_a - \mu_a - v\right)^2}{8\lambda_a v p_m} + \cdots,$$

we obtain the approximation

$$\begin{aligned} p_a &\approx \frac{\lambda_a - \mu_a - v + 2\sqrt{\lambda_a v p_m}\left[1 + \frac{\left(\lambda_a - \mu_a - v\right)^2}{8\lambda_a v p_m}\right]}{2\lambda_a} \\ &= \frac{\lambda_a - \mu_a - v + 2\sqrt{\lambda_a v p_m} + \frac{\left(\lambda_a - \mu_a - v\right)^2}{4\sqrt{\lambda_a v p_m}}}{2\lambda_a} \\ &= \frac{\left(\lambda_a - \mu_a - v + 2\sqrt{\lambda_a v p_m}\right)^2 + 4\lambda_a v p_m}{8\lambda_a\sqrt{\lambda_a v p_m}} \\ &= \frac{4\lambda_a v p_m + 4\lambda_a v p_m\left(1 + \frac{\lambda_a - \mu_a - v}{2\sqrt{\lambda_a v p_m}}\right)^2}{8\lambda_a\sqrt{\lambda_a v p_m}} \\ &= \frac{1}{2\lambda_a}\left(\lambda_a - \mu_a - v + 2\sqrt{\lambda_a v p_m}\right). \end{aligned} \tag{18}$$

Plugging this in eq. (16), the survival probability of a population starting from one wildtype individual is

$$\begin{aligned} p_w &\approx -\frac{1}{\lambda_w - \mu_w - u - v}\left[v\frac{\lambda_m - \mu_m}{\lambda_m} + \frac{u}{2\lambda_a}\left(\lambda_a - \mu_a - v + 2\sqrt{\lambda_a v p_m}\right)\right] \\ &= -\frac{1}{\lambda_w - \mu_w - u - v}\left[v\frac{\lambda_m - \mu_m}{\lambda_m} + \frac{u}{2\lambda_a}\left(\lambda_a - \mu_a - v\right) + u\sqrt{\frac{v\left(\lambda_m - \mu_m\right)}{\lambda_a\lambda_m}}\right]. \end{aligned} \tag{19}$$

Appendix B: Evolutionary rescue probability

Substituting eqs. (9), (16) and (19) into eq. (2), the evolutionary rescue probability can be approximated by

$$p_{\text{rescue}} \approx \begin{cases} 1-\exp\left[\frac{N}{\Delta_w-u-v}\left(v\frac{\Delta_m}{\lambda_m}+\frac{u(\Delta_a-v)}{2\lambda_a}+u\sqrt{\frac{v\Delta_m}{\lambda_a\lambda_m}}\right)\right], & 4\lambda_a v p_m > (\Delta_a-v)^2\,, \\ 1-\exp\left[\frac{v\Delta_m N}{\lambda_m\Delta_w}\left(1-\frac{u}{\Delta_a}\right)\right], & \Delta_a<0 \quad \text{and} \quad 4\lambda_a v p_m < (\Delta_a-v)^2\,, \\ 1-\exp\left[\frac{N}{\Delta_w}\left(\frac{u\Delta_a}{\lambda_a}+\frac{uv\Delta_m}{\lambda_m\Delta_a}+\frac{v\Delta_m}{\lambda_m}\right)\right], & \Delta_a>0 \quad \text{and} \quad 4\lambda_a v p_m < (\Delta_a-v)^2\,. \end{cases} \tag{20}$$

Appendix C: Evolutionary rescue time

We first calculate the expected time for the appearance of the first mutant that rescues the cell population. This can occur either through the evolutionary trajectory $wildtype \rightarrow mutant$ or through the trajectory $wildtype \rightarrow aneuploid \rightarrow mutant$. We start with the former.

Assuming no aneuploidy ($u = 0$), we define T_1 to be the time at which the first mutant cell appears that will avoid extinction and will therefore rescue the population. Note that if extinction occurs, that is the frequency of mutants after a very long time is zero, $m_\infty = 0$, then it is implied that $T_1 = \infty$, and vice versa if $T_1 < \infty$ then $m_\infty > 0$.

The number of successful mutants generated until time t can be approximated by an inhomogeneous Poisson process with rate $R(t) = v p_m w_t$, where $w_t = N\mathrm{e}^{\Delta_w t}$ is the number of wildtype cells at time t. Note that

$$\int_0^t R(z)\mathrm{d}z = v p_m N \frac{\exp[\Delta_w t]-1}{\Delta_w} \approx v p_m N t, \tag{21}$$

by integrating the exponential and because $\frac{\exp[\Delta_w t]-1}{\Delta_w} = \frac{1+\Delta_w t+O(t^2)-1}{\Delta_w} = t + O(t^2)$. The probability density function of T_1 is thus $R(t)\exp\left(-\int_0^t R(z)\mathrm{d}z\right)$. Therefore, the probability density function of the conditional random variable $(T_1 \mid T_1 < \infty)$ is $f_1(t) = \frac{R(t)\exp\left(-\int_0^t R(z)\mathrm{d}z\right)}{p_{\text{rescue}}}$.

We are interested in the mean conditional time, $\tau_1 = \mathbb{E}\left[T_1 \mid T_1 < \infty\right]$, which is given by

$$\tau_1 = \int_0^\infty t f_1(t)\mathrm{d}t = \frac{\int_0^\infty tR(t)\exp\left(-\int_0^t R(z)\mathrm{d}z\right)\mathrm{d}t}{p_{rescue}} = \frac{\int_0^\infty \exp\left(-\int_0^t R(z)\mathrm{d}z\right)\mathrm{d}t}{p_{rescue}} \tag{22}$$

after applying integration by parts. Therefore, plugging eqs. (21) and (2) in eq. (22),

$$\tau_1 = \frac{\int_0^\infty \mathrm{e}^{-vNp_m\frac{\mathrm{e}^{\Delta_w t}-1}{\Delta_w}}\mathrm{d}t}{1-\left(1-p_w\right)^N} \approx \frac{\int_0^\infty \exp\left(-v p_m N t\right)\mathrm{d}t}{1-\mathrm{e}^{-Np_w}} \approx \tag{23}$$

$$\left(1+\mathrm{e}^{-Np_w}\right)\int_0^\infty \mathrm{e}^{-v p_m N t}\mathrm{d}t = \frac{1+\mathrm{e}^{-Np_w}}{v p_m N}, \tag{24}$$

where we use the approximations $\frac{\mathrm{e}^{\Delta_w\tau}-1}{\Delta_w} = \frac{1+\Delta_w\tau+O(\tau^2)-1}{\Delta_w} = \tau + O(\tau^2)$ and $(1-\mathrm{e}^{-Np_w})^{-1} \approx 1+\mathrm{e}^{-Np_w}$ and integrate the exponent. **??**B show the agreement between this approximating and simulation results for intermediate and large tumor sizes.

When $Nu \gg 1$ the aneuploid frequency dynamics is roughly deterministic and therefore can be approximated by

$$a_t \approx \frac{Nue^{\Delta_w t}}{\Delta_w - \Delta_a}\left[1 - e^{(\Delta_w - \Delta_a)t}\right]. \tag{25}$$

As a result, when $N \gg 1$ the number of successful mutants created by direct mutation and via aneuploidy can be approximated by inhomogeneous Poisson processes with the rates

$$r_1(t) = vp_m \int_0^t a_z dz = \frac{uvNp_m}{\Delta_w - \Delta_a}\left(\frac{e^{\Delta_w t} - 1}{\Delta_w} - \frac{e^{\Delta_a t} - 1}{\Delta_a}\right), \tag{26}$$

$$r_2(t) = vp_m \int_0^t w_z dz = vNp_m \frac{e^{\Delta_w t} - 1}{\Delta_w}. \tag{27}$$

For large initial population sizes we assume that the two processes are independent and as a result, they can be merged into a single Poisson process with rate $(r_1 + r_2)(t)$. Consequently, the mean time to the appearance of the first rescue mutant is

$$\tau_2 = \frac{\int_0^\infty e^{-\left(r_1(t)+r_2(t)\right)} dt}{1 - \left(1 - p_w\right)^N} = \frac{\int_0^\infty \exp\left[-\frac{uvNp_m}{\Delta_w - \Delta_a}\left(\frac{e^{\Delta_w t}-1}{\Delta_w} - \frac{e^{\Delta_a t}-1}{\Delta_a}\right) - vNp_m \frac{e^{\Delta_w t}-1}{\Delta_w}\right] dt}{1 - \left(1 - p_w\right)^N}, \tag{28}$$

which we plot in **??**A as a function of the initial population size, N.

We wish to obtain a simpler formula for τ_2, similar to **??**. We thus have the following expansions,

$$\frac{e^{\Delta_w t} - 1}{\Delta_w} = \frac{1 + \Delta_w t + O(t^2) - 1}{\Delta_w} = t + O(t^2),$$
$$\frac{e^{\Delta_a t} - 1}{\Delta_a} = \frac{1 + \Delta_a t + O(t^2) - 1}{\Delta_a} = t + O(t^2),$$

which we use to derive a first-order approximation for τ_2,

$$\tau_2 \approx \left(1 + e^{-Np_w}\right) \int_0^\infty e^{-uNp_m t} dt = \frac{\left(1 + e^{-Np_w}\right)}{uNp_m}, \tag{29}$$