Impact of deleterious passenger mutations on cancer progression

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Cancer progression is driven by the accumulation of a small number of genetic alterations. However, these few *driver* alterations reside in a cancer genome alongside tens of thousands of additional mutations termed *passengers*. Passengers are widely believed to have no role in cancer, yet many passengers fall within protein-coding genes and other functional elements that can have potentially deleterious effects on cancer cells. Here we investigate the potential of moderately deleterious passengers to accumulate and alter the course of neoplastic progression. Our approach combines evolutionary simulations of cancer progression with an analysis of cancer sequencing data. From simulations, we find that passengers accumulate and largely evade natural selection during progression. Although individually weak, the collective burden of passengers alters the course of progression, leading to several oncological phenomena that are hard to explain with a traditional driver-centric view. We then tested the predictions of our model using cancer genomics data and confirmed that many passengers are likely damaging and have largely evaded negative selection. Finally, we use our model to explore cancer treatments that exploit the load of passengers by either (i) increasing the mutation rate or (ii) exacerbating their deleterious effects. Though both approaches lead to cancer regression, the latter is a more effective therapy. Our results suggest a unique framework for understanding cancer progression as a balance of driver and passenger mutations.

chaperones | stochastic simulations | population genetics | unfolding response pathway

Recent advances in sequencing and genotyping of cancer tissues at a genome level have found that individual cancers contain tens of thousands of somatic alterations (1–4). These encompass many genetic alterations, such as single-nucleotide substitutions, insertions, deletions, rearrangements, Loss Of Heterozygosity (LOH) events, copy-number alterations, and whole-chromosome duplications/deletions (1); epigenetic alterations (5); and inheritable changes in cell state. It is generally believed that only a few (2–15) of these alterations cause the cancer phenotype, called driver alterations or simply drivers, whereas the overwhelming majority of events in cancer are believed to have nonsignificant phenotypes and are called passenger alterations or simply passengers. Drivers confer advantageous phenotypes to neoplastic cells (i.e., phenotypes that allow cells in the population to proliferate further). This property is inferred by their effect on cancer-related pathways; frequent occurrence at the same genes, loci, or pathways in different patients (3, 4, 6); and by the structure of cancer incidence rates (7). Because driver events are so critical to cancer progression, their discovery has been the primary goal of genome-wide cancer sequencing (8).

Conversely, little attention has been paid to passengers, which constitute the vast majority of observed somatic alterations in cancer (Table 1) (4). These alterations are assumed to be phenotypically neutral in cancer cells because they are nonrecurrent and are dispersed across a cancer genome (8, 9); however, their phenotype has never been systematically tested. If passengers arise as random alterations, then many can be deleterious to cancer cells (10–12), potentially via proteotoxic stress (13, 14), loss of function (15), provoking an immune response (16), or other mechanisms. Though highly deleterious passengers are weeded out by negative selection, moderately deleterious passengers can evade negative selection and accumulate by mutation-selection balance, ratcheting, or similar mechanisms studied in population genetics (17). Because cancer genomes contain hundreds to thousands of accumulated protein-coding passengers, they may individually exert small effect, yet collectively be significant enough to alter the course of cancer progression.

Here we investigate the possible role of deleterious passenger alterations in cancer progression and examine their potential as an unexploited therapeutic target. First, using an evolutionary model, where passengers can arise alongside drivers in cancer cells, we find that moderately deleterious mutations evade purifying selection and accumulate. The accumulation of passengers alters the dynamics of cancer progression and may explain several clinical phenomena, such as slow progression, long periods of dormancy, the prevalence of small subclinical cancers, spontaneous regression, and heterogeneity in growth rates. These phenomenon cannot be easily explained without considering deleterious passengers. Unlike the current driver-centric paradigm of cancer progression, our analyses demonstrate that progression depends on drivers overcoming passengers. Second, we test the model's predictions by analyzing somatic mutations sequenced in cancers. This analysis shows that, in agreement with the model, individual passengers are likely to be damaging to cells and have largely evaded negative selection. Third, we use our model to explore two possible therapeutic approaches that target passengers and find that increasing either the mutation rate or the deleterious effect of passengers leads to cancer meltdown. The latter therapy may be possible by targeting pathways that buffer the effects of mutations, e.g., unfolded protein response (UPR) pathways. Finally, we present and discuss clinical and biological evidence that supports an important role of passenger alterations in cancer.

Results and Discussion

Evolutionary Model of Cancer Progression Incorporating Passengers. Existing evolutionary models of cancer progression have several limitations. Many models have considered a population of a constant or externally controlled size (18, 19), which does not depend on the absolute fitness of cells. Other models study exponentially growing cancer populations (7, 19, 20), whereas logistic-like behavior has been observed in cancer (21). Most importantly, the vast majority of cancer models (with the exception of ref. 22; see below) neglect the effects of passenger alterations.

Table 1. Passenger mutations in whole-genome sequences

Cancer(s)	Protein-coding mutations	Driver mutations*	Ref(s).
11 breast	115.4	5.1	(4)
10 colon	75	4	(4)
4 astrocytomas	206	5.5	(52)
Acute myeloid leukemia	10	2	(53)
26 melanomas	366	4	(1, 32)
Small-cell lung	100	4	(2)

In most tumors, hundreds of protein-coding mutations accrue, yet only a few are putative drivers. These values are consistent with our model's results. Deleterious passengers may be most exploitable in carcinomas, because leukemia and many blood cancers are generally more sensitive to DNA damage and have earlier incidence rates.
*Classified as drivers by COSMIC (8).

In our stochastic model, individual cells can divide, potentially acquiring driver or passenger alterations, and die. Population size changes with the birth and death of individual cells (Fig. 1A). Generally, the birth and death rates of a cell depend on the effect of accumulated drivers and passengers, and the environment. Assuming that all drivers/passengers possess equal fitness advantage/disadvantage, the birth and death rates $B(d,p,N)$ and $D(d,p,N)$ of each cell depend on the number of drivers d, the number of passengers p, and the total hyperplasia or population size N. Driver mutations increase population size by either increasing the birth rate (e.g., an activating mutation in *KRAS*) or by decreasing the death rate [e.g., a *TP53* knockout that diminishes contact inhibition (23) and apoptosis]. Though specific drivers and passengers will have differing effects on the birth and death rates, we find that aggregating the effects of mutations into the birth rate, and placing the effects of population size into the death rate, does not alter population dynamics from models where mutational effects are split between the two (*SI Appendix*, Fig. S1). Thus, we use

$$B(d,p) = \frac{(1+s_d)^d}{(1+s_p)^p} \qquad D(N) = \frac{N}{K}$$ [1]

where s_d is the fitness advantage (selection coefficient) of a driver, s_p is the fitness disadvantage conferred by a passenger, and K is the initial equilibrium population size—reflecting the effects of the tumor microenvironment. This model assumes multiplicative epistasis and is equivalent or similar to other possible forms (*SI Appendix, SI Text*), which all exhibit qualitatively similar behavior

(*SI Appendix*, Fig. S1). We also let $D(N) = log(1 + N/K)$, for large cancers (grown to 10^6 cells). For small N/K this reduces to the linear model above [similar to previous neoplastic (24) and ecological (25) models], but for large N/K this recapitulates Gompertzian dynamics observed experimentally for large tumors (26). The death rate's dependence on population size is a coarse approximation of many size-dependent factors that tumors must overcome as they expand via additional drivers: contact inhibition, competition between cells for space and resources (e.g., due to a limited crypt size), homeostatic pressure, hypoxia, angiogenesis, limited paracrine signaling, and immune/inflammatory responses to larger tumors (16).

We model cancer progression as a stochastic system of birth (with or without mutations) and death events with defined reaction rates using a standard Gillespie algorithm (27). The system is fully defined by five parameters: s_p, s_d, μT_p, μT_d, and K, where μ is the mutation rate and $T_{d/p}$ are the mutation target sizes for drivers/passengers. Though driver and passenger alterations take many forms, we parameterized our model using single-nucleotide substitution data, as these mutations have been more thoroughly quantified. Because of extensive cancer heterogeneity and limited quantitative knowledge, we varied all parameters by 2–3 orders of magnitude. The ranges we explored centered on values obtained from the literature (*SI Appendix*, Table S1). The mutation rate ($\mu \sim 10^{-8}$ nt^{-1} × division^{-1}; range 10^{-10}–10^{-6}) approximates cells with a mutator phenotype (28). Our initial equilibrium population size ($K \sim 10^3$ cells; range 10^2–10^4) was estimated from hyperplasias within a mouse colonic crypt observed 2 wk after an initiating *APC* deletion (29). The target size for drivers ($T_d \sim 700$ nt; range 70–7,000) approximately 10 potential hotspot mutations per gene (oncogene or tumor suppressor) times 70 driver genes (4). This value was used in previous simulations (19) and is close to the 571 loci with recurrent mutations in colon cancer (30). The target size for functional (nonsynonymous) passengers ($T_p \sim 5 \times 10^6$ nt; range $5 \times 10^5 - 5 \times 10^7$) was estimated as 10^3 nonsynonymous loci per gene times 5,000 well-expressed, non–cancer-related genes in cancer (9). This value is comparable, but less than, a previous estimate of 10 million deleterious loci in cancer (31); does not attempt to capture the 10^4–10^5 noncoding passenger mutations per cancer genome (2, 32); and yet is thousands of times greater than T_d. The chosen driver strength [$s_d \sim 0.1$ (i.e., 10% growth increase per driver); range 0.01–1] was shown to be congruent with cancer onset (19). Passenger deleteriousness ($s_p \sim 10^{-3}$; range 10^{-1}–10^{-4}) was estimated from to the effects of near-neutral germ-line mutations in humans (33) and randomly introduced mutations in yeast (14). Simulations where drivers or passengers conferred a distribution of s_p and s_d did not significantly differ from our fixed-effect
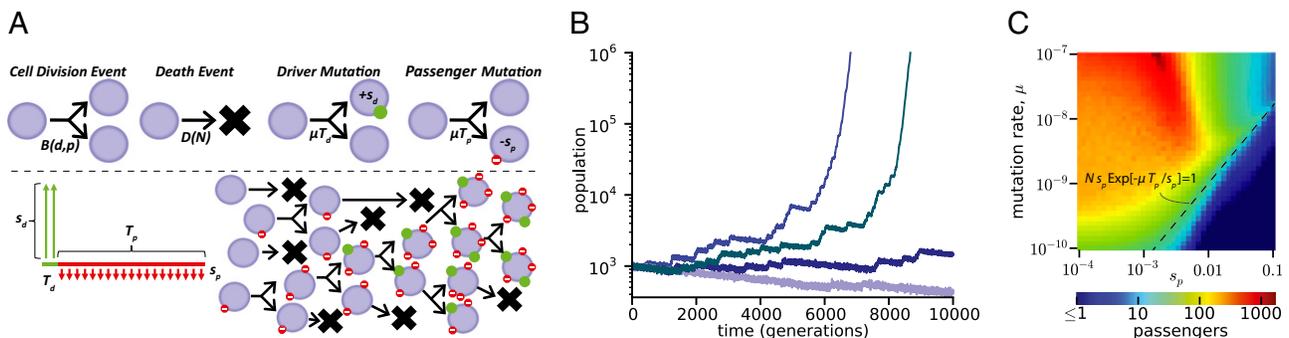
Fig. 1. Dynamics of cancer progression. (*A*) Our evolutionary model: individual cancer cells stochastically divide (potentially acquiring new drivers/passengers) and die. A new driver increases the birth rate by s_d, whereas a passenger decreases it by s_p (Eq. 1). Drivers arise rarely, but have large effects, while passengers are common, but have small individual effects. (*B*) Simulated cancer progression using a Gompertz death rate; despite identical parameters, trajectories exhibit markedly different behavior, sometimes regressing to extinction or having long periods of dormancy. (*C*) The number of accumulated passengers increases with mutation rate and depends, nonmonotonically, on passenger strength.

model (*SI Appendix*, Fig. S1 and discussed below), suggesting that this fixed-effect model adequately captures cancer dynamics. We find that deleterious passengers accumulate under a broad range of conditions (*SI Appendix*, Fig. S2).

We consider death to be any process that prevents a cell from replicating indefinitely, i.e., necrosis, apoptosis, senescence, or differentiation. Thus, N represents only cells capable of infinite division and of carrying the (epi)genetic information in cancer. For this reason, our model lacks asymmetric cell divisions, as this yields differentiated cells. Because we explored the initial population size across two orders of magnitude, our model applies equally well to tumor subtypes dominated by only a small cohort of cancer stem cells and subtypes where cancer may arise from progenitor cells (34). Our model ignores the spatial structure of cancer. Previous studies of asexual populations suggest that ignoring spatial structure will (*i*) underestimate the time for beneficial drivers to sweep through the population and hence the degree of clonal interference, and (*ii*) overestimate the effective strength of selection, which only acts at the geographic boundary between clones (35, 36). Hence, models considering spatial structure should find that more passengers fixate relative to those that do not, strengthening the conclusions of our model.

Moderately Deleterious Passengers Fixate and Alter Cancer Progression.
Fig. 1*B* presents typical population trajectories of cancer beginning at the first driver mutation. All trajectories consist of intervals of rapid growth and gradual decline. A new driver leads to a clonal expansion of the subpopulation carrying this driver, causing short periods of rapid growth. Growth stops when the effect of this driver is balanced by the death rate, which increases with population size. While the population waits for the next driver to arise, passengers steadily accumulate, causing a gradual decline of population size. Together, these processes cause trajectories to grow in a sawtooth pattern.

Simulated tumors exhibit either unconstrained growth or regression, often after a period of dormancy (Fig. 1*B*). We find that the probability of either outcome depends on the tumor size: tumors larger than a critical size ($N_{critical}$) are likely to progress, whereas smaller tumors are likely to regress (*SI Appendix*, Fig. S3). Indeed, larger populations acquire drivers more frequently, as they have more cells in which drivers can arise. Moreover, natural selection weeds out deleterious mutations more efficiently in larger populations (Fig. 2*B*). The phenomena of dormancy and spontaneous regression, observed both in our model and clinically (37), do not occur in models lacking deleterious passengers. In *SI Appendix, SI Text*, we estimate $N_{critical}$ for cancer and provide a framework for understanding where deleterious passengers are most relevant (*SI Appendix*, Fig. S4).

Importantly, simulations show that hyperplasias that progress to clinical size (i.e., 10^6 cells, 15–20 drivers) accumulate many deleterious passengers. Evasion of purifying selection and fixation of deleterious passengers is an unexpected result not programmed into the model. Although the exact number of accumulated passengers depends on μ and s_p (Fig. 1*C*), 10^2–10^3 deleterious passengers are obtained for a broad range of parameters, consistent with the numbers of nonsynonymous substitutions observed in cancer genomics studies (Table 1), suggesting that observed passengers in sequencing data can be moderately deleterious.

We then studied how deleterious mutations can accumulate despite negative selection. Previous studies have calculated the rate of accumulation of deleterious mutations in the absence of clonal expansions (38–40). We identified two previously known processes that allow passengers to evade negative selection in cancer: hitchhiking alongside a driver and Muller's ratchet (25) (Fig. 2). Deleterious passengers hitchhike when the cell they reside in acquires a new driver, which then leads to a clonal expansion and fixation of all the mutations in that cell. Muller's ratchet, in turn, is a process of gradual accumulation of deleterious mutations and population decline in the absence of drivers. In Muller's ratchet, a mutation-selection balance arises after driver sweeps, which creates a steady-state Poisson distribution of the
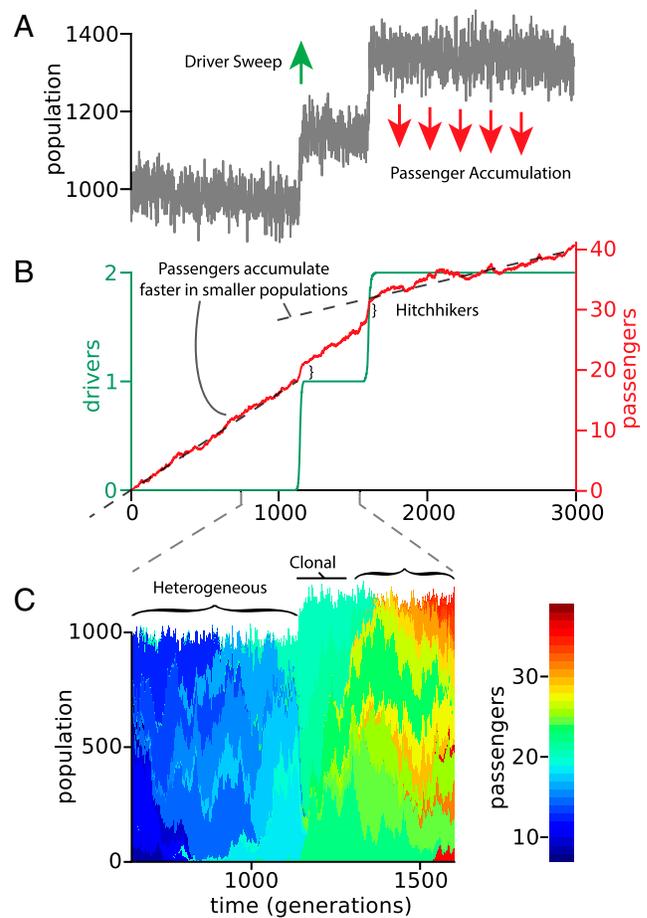
Fig. 2. Mechanisms of passenger accumulation. (*A*) Spurts of population growth, caused by the acquisition of a new driver, are interspersed with a gradual decline due to passenger accumulation. (*B*) Passengers accumulate both steadily between the arrival of drivers and by hitchhiking during clonal expansions. (*C*) Each subclone, containing a unique number of passengers (shown by color), grows and declines stochastically, eventually to extinction. In between drivers, the population becomes heterogeneous. A new driver will promotes only one clone, creating a clonal population. Afterward, new mutations on top of the previous hitchhikers restore heterogeneity.

number of passengers per cell with mean and variance $\mu T_p/s_p$ (first described in ref. 41; *SI Appendix, SI Text* and Fig. S5). The fittest subpopulation—cells with the fewest passengers: $\sim N\mathrm{Exp}[-\mu T_p/s_p]$ cells—is much smaller than the whole population, so it can spontaneously shrink to extinction (Fig. 2*C*). When back mutations are rare, such an extinction leads to the irreversible loss of this least-mutated fraction of cell and corresponds to a "click" of Muller's ratchet (25). This process is especially rapid during clonal expansions when the size of the expanding clone is small. Both of the above processes, well known in population genetics, are augmented in cancer because of the presence of strong drivers.

Simulations show that moderately deleterious, rather than highly deleterious or neutral, passengers have a major effect on cancer progression (Fig. 3*A*). Indeed, almost-neutral passengers have very little effect on cancer cells, and passengers of large effect do not accumulate (31). By slowing progression to cancer, moderately deleterious passengers accumulate in even greater numbers than neutral mutations despite their slower accumulation rate (Fig. 3*A*). Importantly, we find that moderately deleterious passengers affect progression for s_p from 10^{-3} to 2×10^{-2}, which subsumes the best literature estimates of the strength of
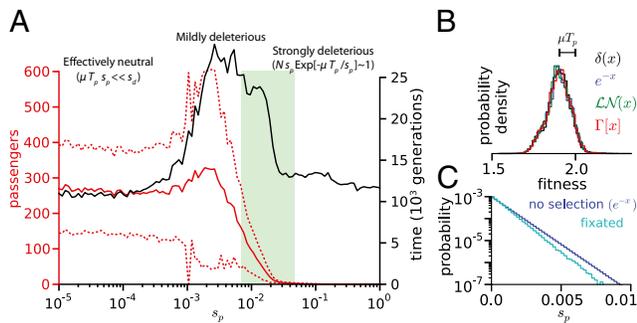
Fig. 3. Moderately deleterious passengers alter cancer progression and mostly evade selection. (*A*) Passengers of intermediate fitness effect s_p prolong the time to cancer and accumulate in large, highly variable quantities (red solid, mean; dotted, ±1 SD). Moderately deleterious passengers affect cancer only if they are strong or frequent enough to be comparable to the effects of drivers, yet weak enough to avoid selection (*SI Appendix, SI Text*). Experimentally observed fitness effects of random point mutations in YFP in yeast ranged from 0.007 to 0.028 (green shading) (14). (*B*) Population dynamics did not change noticeably when passengers were drawn from various distributions of fitness distributions (*SI Appendix, SI Text*). (*C*) Passenger fixation probability declined only moderately with increasing deleteriousness.

deleterious mutations (14, 33). Such small selection coefficients for individual passengers are typically undetectable in cell cultures, yet critical for long-term cancer dynamics.

We then relaxed our assumption that s_p is constant for all passengers, by simulating cancer progression with passengers drawn from distributions of deleteriousness (*SI Appendix*, Fig. S6). The strength of driver and passenger mutations affects their fixation probability (Fig. 3 *B* and *C*). For passengers, the variation in fitness within a population is mostly invariant to the type of distribution of passenger effects (Fig. 3*B*). Negative selection against passenger fixation appears to be largely inefficient, except for highly deleterious passengers (Fig. 3*C*).

The significant variance in cell fitness within the population, caused by deleterious passengers (Fig. 3*B*), also affects the probability of driver fixation. Because a driver will generally occur in a cell of average fitness, it is unlikely to fixate unless its new fitness is greater than the fittest cells. The difference between the fittest cells and average cells in the population is approximately μT_p and independent of s_p (Fig. 3*B*) (17); therefore, a driver must confer a benefit greater than μT_p to fixate (*SI Appendix*, Fig. S1). This argues that weak drivers are unlikely to fixate in cancer or be observed in genomic sequencing.

In summary, our simulations demonstrate that despite the moderately deleterious effect of individual passengers, they accumulate in large numbers during neoplastic progression, reducing the fitness of cancer cells and altering the course of neoplastic progression. We find several reasons why deleterious passengers accumulate more than might be expected a priori: (*i*) mutator phenotypes [a hallmark of cancer (28)] accelerate accumulation rates; (*ii*) small population sizes in the early stages of cancer progression enhance accumulation rates; (*iii*) driver-induced bottlenecks and hitchhiking contribute additional passengers; (*iv*) passengers prolong progression—offering more time for accumulation; and (*v*) passengers arising as part of a distribution of deleteriousness fixate more often than equivalent passengers considered in isolation. These first three phenomenon, though undocumented in cancer theory, have been previously observed in population genetics (12).

Passenger Mutations Observed in Cancer Can Be Damaging. Our model makes several testable predictions: (*i*) accumulated passengers in cancer populations can be deleterious to cancer cells; (*ii*) the deleterious effect of an individual passenger has little bearing on its likelihood of accumulation; and (*iii*) fixed

drivers should have larger effects on phenotype than passengers. Cancer genomics data provide an opportunity to test these predictions. First, we test whether nonsynonymous passengers found in cancer are damaging or neutral to protein function using comparative genomics. Second, we test whether selection acting against passengers is effective at preventing fixation or largely ineffective, as suggested by our simulations.

We analyzed 116,977 cancer mutations curated by the Catalogue of Somatic Mutations in Cancer (COSMIC) and The Cancer Genome Atlas (TCGA). We classified them as driver and passenger mutation groups and then characterized their effects using PolyPhen, a tool widely used in population and medical genetics to predict the damaging effect of missense mutations (15). Passengers were identified as missense mutations that show no recurrence and affect genes not listed in a census of possible cancer-causing genes (*SI Appendix, SI Text*). The $\Delta PSIC$ metric of PolyPhen measures the degree of evolutionary conservation of a mutated residue (42) by calculating the negative log-likelihood of observing a specific mutation, given the evolutionary history of the protein. Specifically, a mutation with a $\Delta PSIC$ of 1 is $e(= 2.71...)$ times less likely to be observed than the wild-type allele, as computed from a multiple alignment. Thus, a mutation with high $\Delta PSIC$ is more likely to be damaging to molecular function (43) because this implies the mutation disrupts a well-conserved residue. PolyPhen has been extensively tested and benchmarked (15).

Fig. 4 presents this analysis for passengers, drivers, and three reference datasets: (*i*) common human missense SNPs; (*ii*) simulated de novo mutations (randomly generated using a cancer-specific three-parameter model; *SI Appendix, SI Text*); and (*iii*) damaging, pathogenic missense mutations causing human Mendelian diseases (from the Human Gene Mutation Database). As expected, common SNPs are benign and exhibit small $\Delta PSIC$ values, whereas disease-causing mutations, with known damaging effect, exhibit large $\Delta PSIC$ values (Fig. 4*A*). Driver mutations exhibit similarly high values of $\Delta PSIC$, significantly greater than randomly generated mutations, indicating that drivers tend to occur at well-conserved loci. From a biochemical perspective, this result shows that, to activate an oncogene or to disable a tumor suppressor, the driver mutation must change a critical and well-conserved residue, e.g., the GTP binding site of Ras or DNA binding domain of p53. From an evolutionary perspective, the conservation of residues that promote tumorigenesis when mutated suggests strong natural selection against the early development of cancer. The ability of $\Delta PSIC$ score to identify drivers as having highly nonneutral phenotypes (i.e., damaging or altering
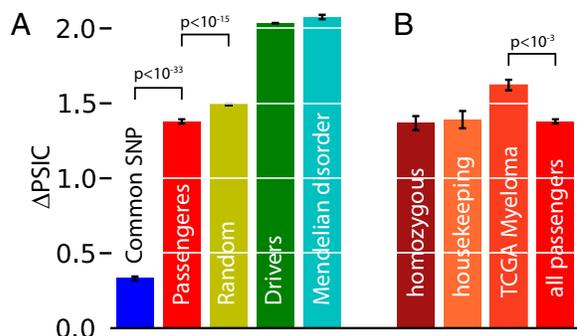
Fig. 4. Characterization of missense mutations in cancer sequencing data. (*A*) Mutations were assayed using the $\Delta PSIC$ score of PolyPhen, which estimates the damaging effect of a new mutation, given known homologs; mutations with high $\Delta PSIC$ scores are most likely damaging (43). Passengers have large $\Delta PSIC$, close to random mutations, suggesting that they are deleterious. (*B*) Deleterious passenger phenotypes were observed in all subsets of passengers studied, arguing that these results cannot be explained by recessive phenotypes, or lack of expression, or database biases.

molecular function) validates its use for characterizing somatic cancer mutations. The exceptionally high $\Delta PSIC$ scores for these mutations are consistent with our third prediction that drivers must be of strong effect.

Most importantly, passenger mutations exhibit $\Delta PSIC$ values that are on average much greater than neutral mutations (Fig. 4A; $P < 10^{-33}$); therefore, many passengers affect conserved residues and are likely damaging to protein function. This result clearly demonstrates that passenger mutations are nonneutral. To ensure that our set of putative passenger mutations was not contaminated by drivers, we increased our stringency of passenger classification, but found no statistically significant change ($P = 0.69$) in mean $\Delta PSIC$ (*SI Appendix, SI Text*); additional safeguards are explored below.

Passengers exhibit $\Delta PSIC$ values much lower than drivers (Fig. 3A), supporting the assumption of our evolutionary model that deleterious passengers are generally much weaker than drivers ($s_p << s_d$). The $\Delta PSIC$ values of passengers are close to, but lower than, values of randomly generated mutations (Fig. 3A; $P < 10^{-15}$), suggesting that many passenger mutations evade purifying selection. Still, a statistically significant difference between these two sets demonstrates slight negative selection against the most deleterious passengers. This comparison of passengers and random mutations fully supports our model's prediction that selection against moderately deleterious passengers is largely ineffective in neoplastic progression (Fig. 3C).

To rule out possible caveats where passengers have damaging effects on protein function but no effect on the fitness of cancer cells, we performed additional tests. For example, passengers with deleterious scores could affect only genes that are functionally unimportant or not expressed in cancer cells. Thus, we considered only passengers in essential and ubiquitously expressed housekeeping genes, but still observe equally high $\Delta PSIC$ scores (Fig. 4B). This eliminates the possibility that damaging passengers are not expressed or present in unimportant genes. Alternatively, perhaps only recessive heterozygous passengers exhibit high $\Delta PSIC$ scores; if so, cell fitness would remain unchanged because the other allele provides sufficient functionality. We observe equally high $\Delta PSIC$ scores for homozygous passengers (which can arise via LOH events or chromosomal losses), refuting this possibility (Fig. 4B). Collectively, our analyses show that signatures of damaging mutations are ubiquitous in known passengers and likely affect the fitness of cancerous cells.

As an alternative powerful test, we assayed for signatures of selection in driver and passenger genes by comparing the observed ratio of nonsynonymous to synonymous mutations (ω) to the predicted ratio using a random model of mutations (*SI Appendix, Fig. S7*). This distribution reaffirmed COSMIC's driver and passenger classifications. Genes with $\omega < 1$ likely experience purifying selection and these genes were generally classified as passengers by COSMIC. Conversely, genes with $\omega > 1$ likely experience positive selection and were nearly all classified as drivers by COSMIC. Most importantly, the shape of this distribution corroborates the narrative of a few strong drivers overlaid with copious passengers experiencing nearly undetectable negative selection that we observe in both our modeling and $\Delta PSIC$ analysis: A total of 94% of genes had an observed $\omega < 1$, and their occurrence was only very moderately enriched relative to our neutral model—on the fringe of statistical significance: $P = 0.012$—and not nearly as pronounced as the signal for drivers. The rare driver genes, with $\omega > 1$, often exhibited extreme nonsynonymous substitution rates vastly greater than expected from a neutral model of evolution: ω of *KRAS*, *TP53*, *BRAF*, and *PTEN* were all greater than 40.

Though our genomic analysis of passenger mutations focused on missense substitutions, our model is generalizable to all inheritable (epi)genetic alterations, including those that are present at low frequency in the cancer population. Indeed, the length distribution of somatic copy number alterations (SCNAs) in cancer suggests these alterations are under purifying selection as well (44). Hence, the total load of accumulated deleterious passengers in cancer may be greater than that estimated from single nucleotide mutations detected in genome sequencing.

Accumulated Passenger Mutations Can Be Exploited for Cancer Treatment. Using our evolutionary model, we probed how cancers that accumulated passenger alterations respond to passenger-centric treatments. We tested two strategies: (*i*) increasing the overall mutation rate (μ), thus increasing the rate of passenger accumulation, and (*ii*) magnifying the deleterious effect of passengers (s_p), as described below. Both strategies reduce cancer size (Fig. 5); however, mutagenic strategies require more severe increases (~50-fold) in the mutation rate to succeed (Fig. 5A), whereas fivefold magnifications of deleterious effect suffice. Even with large mutation rate increases, the probability of 5-y relapse following treatment initiation is considerable (Fig. 5B). This behavior resembles patient responses to existing chemotherapeutic agents that elevate mutation rates.

In practice, increasing the deleterious effect of passengers, both mutations and chromosomal alterations (45), could be achieved by inhibiting cellular mechanisms that buffer against the effects of mutations or incorrect protein dosage (14). Hence, deleterious effect could be increased by targeting chaperones, proteasomes, or other components of UPR pathways (46); or by elevating ER stress (47); or by stimulating protein misfolding using hyperthermia (48). These passenger-mediated therapies should specifically affect cancer cells because somatic mutations are generally rarer in normal tissues (9). For example, a recent study of clonal mosaicism in human brains found only 1.5 SCNAs per adult sample (49), whereas a recent pan-cancer survey found 42 SCNAs per cancer (3).

Several experiments support this strategy of exacerbating passengers' effect. First, chaperones are widely expressed in cancer, indicative of poor prognosis (50), and their inhibition (or proteasome inhibition) exhibits antitumor activity (46). Though other specific roles of chaperons and proteasomes in cancer were proposed, our framework suggests that cancers buffer against the effects of passenger alterations using UPR machinery. In our paradigm, inhibiting the UPR unleashes the effects of accumulated
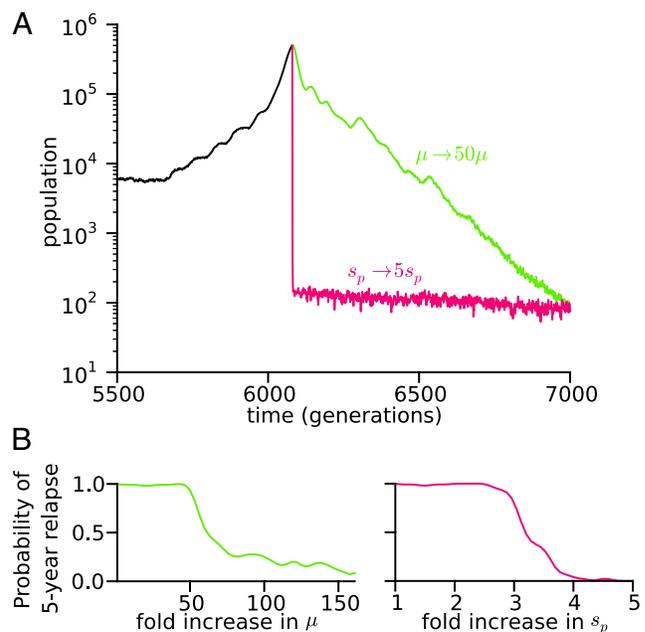
Fig. 5. Deleterious passengers can be exploited for treatment. (*A*) Cancers grown to 10^6 cells are treated by increasing the mutation rate (green) or deleterious effect of passengers (magenta). Both strategies lead to reduction in cancer size. (*B*) Much smaller increase of the deleterious effect of passengers is sufficient to prevent 5-y relapse.

passengers. Recent discoveries that aneuploidy and chromosomal imbalance lead to proteotoxic stress (45) and a dependence on the UPR for survival (47), and that very high levels of DNA damage correlate with better clinical outcomes (43, 51) (a paradoxical result in the classical paradigm of cancer), are consistent with our framework.

One of the major limitations of driver-targeted therapies is that they can be defeated by a cancer's ability to rapidly evolve resistance by acquiring new mutations. Our approach, of increasing the deleterious effects of passengers, is different as it targets not only existing cancer cells, but also cancer's ability to accumulate new mutations and thus its evolvability.

Cancer research has focused primarily on driver alterations with little attention to the overwhelming majority of potentially harmful passenger alterations that arise along the way. To the best of our knowledge, this "dark matter" of cancer genomes has not yet been explored. We developed an evolutionary model of cancer progression that clearly demonstrates that deleterious passengers can accumulate in cancer, while our genomic analysis confirms that passengers presented in sequenced cancers have

damaging phenotypes. Importantly, considering cancers as a balance between drivers and deleterious passengers reproduces many observed phenomena in cancer, including (*i*) slow initial and rapid late growth; (*ii*) a critical cancer size for dormancy or spontaneous regression; and (*iii*) short-term response to mutagenic therapies (*SI Appendix*, Table S2). These phenomena were not preprogrammed into the model, suggesting that the deleterious effect of passengers explains many properties of cancers.

Materials and Methods

Simulations were executed using the Next Reaction (27), a Gillespie algorithm. All cancer mutations were collected from COSMIC version 42 (30). See *SI Appendix, SI Text* for details.

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EVOLUTION