and vehicle control treated mice (n=5 mice/group). Lines in (**f**) are mean±s.e.m (two-tailed Mann-Whitney test). Black dots in (**g**) show experimental data. (**h-i**) Numerical example (see **Supplementary Note**) of the model showing increased proportion of tumors resistant to displacement by mutant clones (**h**) and the decrease in tumor density following DEN-treatment (**i**). Experimental data depicts mean±s.e.m. (**j**) Images of human (top) and mouse (bottom) esophagus. Dotted lines delineate lesions. (**k**) Confocal images of human normal (top) and neoplastic (bottom) esophageal epithelium stained with KRT6 (red) and Topro3 (nuclei, blue). Scale-bars: 100μm. Simulations in (**a, g, h and i**) show the mean and range between the minimum-maximum outputs of the model run with the accepted parameters from Approximate Bayesian Computation (**Methods**).

Supplementary Tables

This file contains **Supplementary Tables 1-11**. **Supplementary Table 1** lists the area (μm^2) and number of tumors measured at different time points post-DEN treatment. **Supplementary Table 2** displays the targeted sequencing data of isolated tumors from 10 days post-DEN treated mouse esophageal epithelium. **Supplementary Table 3** displays the targeted sequencing data of isolated tumors from 1 year post-DEN treated mouse esophageal epithelium. **Supplementary Table 4** shows the dN/dS results from targeted sequencing data of isolated tumors from 10 days post-DEN treated mouse esophageal epithelium. **Supplementary Table 5** shows the dN/dS results from targeted sequencing data of isolated tumors from 1 year post-DEN treated mouse esophageal epithelium. **Supplementary Table 6** shows the WES results of 9 and 18 month post-DEN tumors. **Supplementary Table 7** displays the dN/dS results for WES of 9 and 18 month post-DEN tumors. **Supplementary Table 8** displays the HGFP mean intensity in tumors and surrounding normal esophageal epithelium. **Supplementary Table 9** shows the targeted sequencing data of normal esophageal epithelium from 10 day post-DEN treated mice. **Supplementary Table 10** displays the dN/dS results from targeted sequencing data of normal esophageal epithelium from 10 day post-DEN treated mice. **Supplementary Table 11** lists the parameters for the mathematical model.

Supplementary Videos

Supplementary Video 1: Esophageal epithelium at 1 month post-DEN. Mouse esophageal epithelium at 1 month post-carcinogen treatment. The tissue was stained with Dapi (blue) and KRT6 (red) and imaged by confocal microscopy. Individual images were taken using a 10x objective and merged to create a 3D single cell resolution image of the entire esophagus. The video initially shows the entire tissue before zooming in on a tumor, characterized by an irregular nuclear distribution and increased KRT6 expression.

Supplementary Video 2: Early angiogenesis in a 10 days post-DEN tumor. Mouse esophageal epithelium at 10 days post-carcinogen treatment. The tissue was stained with Dapi (blue), KRT6 (red) and CD31 (yellow, to label endothelial cells) and imaged by confocal microscopy using a 40x objective. The video shows small capillary circling the tumor (characterized by an irregular nuclear distribution and increased KRT6 expression), illustrating the early steps of angiogenesis.

Supplementary Video 3: Angiogenesis in an established tumor. Mouse esophageal epithelium at 9 months post-carcinogen treatment. The tissue was stained with Dapi (blue), KRT6 (red) and CD31 (yellow, to label endothelial cells) and imaged by confocal microscopy using a 40x objective. The video shows a developed vasculature surrounding the established tumor (characterized by an irregular nuclear distribution and increased KRT6 expression).

Supplementary Note: This document sets out the theory and mathematical modelling of tumour dynamics in Sections 1–7. Section 1 discusses previous results on the growth and competition of mutant clones in normal oesophageal epithelium. Section 2 describes a previously proposed stochastic model of tumour dynamics. Section 3 describes the elimination of tumours by highly competitive mutant clones in the surrounding normal epithelium. Section 4 shows how reducing the competitive imbalance between tumours and highly fit mutant clones in the normal tissue affects tumour survival. Section 5 describes the selection pressure on tumours from competition with surrounding clones in the normal epithelium. In Section 6, we substitute simple mathematical equations into the model to numerically illustrate the principles described in the previous sections. Section 7 is a summary of our conclusions.

Supplementary Material for

Mutant clones in normal epithelium outcompete and eliminate emerging tumours

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Theory and mathematical modelling

Modelling aims

Here we describe a general model of tumour loss that allows us to make testable predictions without requiring details of cell dynamics in tumours or mutant clones in the epithelium. We are interested in particular in whether the tumours are lost due to tumour-intrinsic mechanisms, or if the tumours are removed by mutant clones in the adjacent normal epithelium. As we discuss the factors affecting tumour loss, the principles of the model are described in mathematical terms so that we can later construct a numerical version of the model. This numerical version of the model is intended as a demonstration of the model principles, rather than a parameter- or model-fitting exercise.

In **Section 1**, we briefly summarize previous results regarding growth and competition of mutant clones in normal oesophageal epithelium. In **Section 2**, we describe a previously proposed stochastic model of tumour dynamics and apply it to the tumour density following DEN-treatment. This model shows how tumour loss might occur due to the stochastic nature

of the cell dynamics in oesophageal epithelium. However, it does not consider any interaction between tumour cells and the surrounding normal tissue. In **Section 3**, we show that tumour loss is increased by the induction of highly fit mutant clones in the normal epithelium. This justifies incorporating into the model the effects that mutant clones surrounding the lesions could have in tumour loss. To further challenge this hypothesis, in **Section 4**, we show how reducing the competitive imbalance between tumours and highly fit mutant clones in the normal tissue increases the number of surviving tumours. If competition with surrounding clones can remove early neoplasms, we might expect this competition to act as a selective pressure on tumours. This is the topic of **Section 5**, where we discuss how if certain tumour genotypes reduce the competitive imbalance between the tumour and mutant clones in the surrounding tissue, those tumour genotypes will have a higher chance of survival and will become enriched in the tumour population. In **Section 6**, we substitute simple mathematical equations into the model to numerically illustrate the principles described in the previous sections. We show how the various experimental results in this study are all consistent with this model in which mutant clones in the normal tissue contribute to the loss of tumours. **Section 7** is a brief summary of our conclusions.

1. Clonal competition in oesophageal epithelium

Before examining the behaviour of tumours in the tissue, we first summarize previously published results relating to competition of mutant clones in phenotypically normal murine oesophageal epithelium[13].

The mouse oesophageal epithelium is maintained by proliferating cells in the basal layer (**Extended Data Fig. 1a**). These cells divide stochastically (randomly) (**Extended Data Fig. 1b**), meaning that, if we track the offspring of basal cells, some cell lineages will grow into multicellular clones of the original cell, and other lineages will be lost as all basal cells differentiate[15]. A mutation in a basal cell may convey a growth advantage, biasing cell fate towards producing more dividing daughter cells and promoting the growth of a mutant clone[11-14,46] (**Extended Data Fig. 1c**).

When the tissue contains multiple mutant clones, the clones compete for the limited space in the tissue[13] (**Extended Data Fig. 1d**). Fitter clones are able to displace less fit clones, but once a clone is surrounded by clones of similar competitive fitness it returns towards more neutral growth[13] (**Extended Data Fig. 1d**). The expansion and survival of a mutant clone therefore depends not only on its own fitness, but the fitness of its neighbours[13].

Following DEN treatment, the density of tumours in the oesophagus falls rapidly (**Fig. 1d**). Tumours are not lost due to apoptosis (**Extended Data Fig. 6a**), abnormal proliferation of tumour cells (**Extended Data Figs. 6b-g**) or elimination by the immune system (**Extended Data Figs. 6h-p**). The tumours grow amongst a dense patchwork of highly fit clones competing for their place in the tissue[13]. In the following sections we explore whether the tumours survival is affected by the mutant clones in the normal epithelium around them.

2. Stochastic model of tumour dynamics

We start by considering an existing model of cell dynamics in mouse oesophageal epithelial tumours to examine whether it is capable of explaining the pattern of tumour loss over time. This model was based on the shallow proliferative-differentiated cell hierarchy that operates in the normal oesophagus[15,16].

In a previous study, oesophageal tumour growth in DEN- and Sorafenib-treated mice was found to be consistent with a stochastic model of cell dynamics[17]. In this model, proliferating cells divide to form a pair of proliferating daughter cells, a pair of non-dividing daughter cells, or one cell of each type. In the normal tissue, the probabilities of each symmetric division type are balanced, so that the total number of proliferating cells remains approximately constant (**Extended Data Fig. 1b)**. In the tumours, there was a bias towards producing more dividing than differentiated cells, causing the average size of tumours to increase over time (**Extended Data Fig. 1c**)[17]. Due to the stochastic nature of the process, some tumours expand in size, while in others all basal cells differentiate and the tumour is shed and lost from the tissue[17,47]. This variation in outcome due to random chance is known as drift.

The stochastic system here parallels the continuous time Markov process used to model the dynamics of mutant clones with imbalanced cell fates in normal epithelium[14,46]. We can therefore modify the equations used in these studies to calculate the probability that a tumour will lose all of its proliferating cells due to stochastic drift. The clones in normal epithelium are assumed to originate from single cells[14,46], so we must alter the equations for this study to allow for the likely possibility that the tumours contain multiple proliferating cells at the end of the DEN treatment.

Asymmetric divisions do not alter the number of proliferating cells, which means we only need to consider the symmetric divisions. Letting P and D represent a proliferating and a differentiated cell respectively, the simplified version of the model shown in **Extended Data Fig. 1c** becomes

$$P \xrightarrow{2r\lambda} \begin{cases} P + P & Prob.\ \frac{1}{2} + \Delta \\ D + D & Prob.\ \frac{1}{2} - \Delta \end{cases}$$

where λ is the division rate, $2r$ is the fraction of symmetric divisions, and Δ is the probability imbalance between symmetric division and symmetric differentiation (**Extended Data Fig. 1c**)[46]. If Δ=0, equal proportions of proliferating and differentiating cells are produced (**Extended Data Fig. 1b)**, as occurs in neutral growth of wild type clones[15]. The value of Δ can range from -0.5 (all symmetric divisions produce two differentiated cells) to 0.5 (all symmetric divisions produce two proliferating cells).

The probability that a cell lineage starting from a single cell will go extinct by time *t*, $\alpha(t)$, is given by [48]

$$\alpha(t) = \frac{\left(\frac{1}{2} - \Delta\right) e^{2dt\Delta} - \left(\frac{1}{2} - \Delta\right)}{\left(\frac{1}{2} + \Delta\right) e^{2dt\Delta} - \left(\frac{1}{2} - \Delta\right)}$$

where d is the symmetric division rate of tumour cells ($r\lambda$).

In this model, each cell is assumed to behave independently of all others, and therefore the extinction probability of a population starting with *n* proliferating cells equals the probability that *n* independent cell lineages starting from single cells all become extinct, i.e. $\alpha(t)^n$. If we assume that fully differentiated tumours are lost from the tissue, then the survival probability of a tumour is given by 1 minus the extinction probability:

$$p_{surv}(t) = 1 - \alpha(t)^n \quad (1)$$

where *n* is the initial number of proliferating cells in each tumour (assumed here to be the same for all tumours). With *n*=1, Eq.1 matches that used in previous studies for the survival probability of clones that originate from single proliferating cells[14,46]. For a full derivation of the equations used here and in the cited studies of imbalanced clone dynamics, see [48].

Fitting equation 1 to the tumour density following DEN-treatment results in a steep initial drop in tumour numbers followed by a slower downward trend, consistent with the experiment (**Extended Data Fig. 10a**). The median accepted parameters found from fitting the model to the data (**see Methods**) were (95% credible interval lower bound, upper bound) d=0.33/day (0.27, 0.36), Δ=0.003 (0.001, 0.005), n=1.2 (1.0, 1.5), and initial tumour density (immediately following DEN-treatment)=4.8/mm^2 (4.1, 5.6), though these parameters should not be interpreted as estimates of the true biological values (**see sections below**).

However, we will see in the sections below that this model is not capable of explaining the results seen in the full range of experiments, and therefore must be rejected (or at least adjusted) to account for the clones in the surrounding tissue.

3. Elimination of tumours by highly competitive mutant clones in the surrounding normal epithelium

Although the stochastic model of tumour drift defined above is sufficient to describe the pattern of tumour loss following DEN-treatment, it does not rule out alternative causes of tumour loss. The normal epithelium surrounding the tumours contains a patchwork of competing mutant clones (**Fig. 2g, Extended Data Fig. 7 and Section 1**). Therefore, we speculated that, like clones in the surrounding normal tissue, tumours may be displaced by highly fit mutant clones.

We considered a general model in which a highly competitive mutation, *M*, is induced in the tissue following DEN treatment. We assume that clones of the mutant *M* are able to remove tumours that they encounter (**Extended Data Figs. 8a and 9g**). Let $p_M(t)$ be the survival probability of a tumour assuming that the mutant *M* is the only cause of tumour loss. Let p_{other} be the survival probability of a tumour based on all other sources of tumour loss (such as drift – see **Section 2**, but may include tumours removed by DEN-created clones – see **Section 4** below). For simplicity, we assume that tumour loss due to the mutant *M* is independent of all other causes of tumour loss. The combined survival probability of a tumour is then given by

$$p_{surv}(t) = p_{other}(t)\, p_M(t) \tag{2}$$

Let *M(t)* be the proportion of tissue covered by the mutant at time *t*. We make the following additional assumptions for the sake of simplicity:

1. The proportion of tissue covered by *M* increases monotonically.
2. Tumours are spread randomly across the tissue.
3. Tumours are removed instantly with probability 1 when the mutant *M* colonizes the location of the tumour (see the end of this section for a discussion of this assumption).

This means

$$p_M(t) = 1 - M(t) \tag{3}$$

and the combined probability of tumour survival is then given by

$$p_{surv}(t) = p_{other}(t)\left(1 - M(t)\right) \tag{4}$$

To make it easier to compare mice in which the initial density of tumours may vary, we looked at the proportion of tumours eliminated (*PTE*) by *M*, using the tumour density in the non-M-mutant regions of the tissue to estimate the tumour density in the full tissue in the absence of *M*.

$$PTE = 1 - \frac{total}{Mneg} \tag{5}$$

where *total* is the density of tumours over the full tissue, and *Mneg* is the density of tumours in the M-negative region.

The expected *PTE* for two models - where the *M* mutant removes tumours it encounters (PTE_M) and where tumour loss is independent of the mutant in the surrounding tissue ($PTE_{\neg M}$), are

$$PTE_M = 1 - \frac{p_{surv}(t)}{p_{other}(t)} = M(t) \tag{6}$$

$$PTE_{\neg M} = 1 - \frac{p_{other}(t)}{p_{other}(t)} = 0 \quad (7)$$

In other words, if *M* clones are able to remove tumours in a similar manner to which highly fit clones are able to displace weaker clones in the normal tissue, then we will see a reduction in tumour numbers proportional to the spread of the *M* mutant. By definition, models in which tumour survival is independent of surrounding tissue ($PTE_{\neg M}$), such as the stochastic tumour drift model described in **Section 2**, predict that tumour numbers will be unaffected by the spread of the *M* clones (**Extended Data Fig. 10b**).

To test these predictions, we used an inducible DN-Maml1 mutation that prevents Notch signalling and forms rapidly expanding clones when induced in murine oesophageal epithelium[11,13]. When DN-Maml1 mutant clones are induced in the normal epithelium, the density of tumours is significantly reduced compared to uninduced control tissues (**Figs. 3b and c**). The density of tumours is not altered in regions of the induced oesophagus which are not occupied by DN-Maml1 mutant clones, and therefore the tumour density reduction is occurring only in the DN-Maml1 mutant areas (**Extended Data Fig. 8f**). Furthermore, as predicted under the assumption that DN-Maml1 clones can remove tumours they encounter from the tissue, the data shows a strong correlation between DN-Maml1 clone spread and tumour loss (**Fig. 3d, Extended Data Figs. 8g and 10b**). The results of the experiment therefore indicate that DN-Maml1 clones are contributing to the loss of tumours from the tissue.

In the experiment, there remained a small number of tumours in close contact with DN-Maml1 mutant regions (**Fig. 3e**). This may indicate that there is a lag time between contact with DN-Maml1 clones and tumour removal and that the surviving tumours seen in the DN-Maml1 areas are in the process in being removed (**Fig. 3e and Extended Data Figs. 9a to c**). The clear significance of the experimental results and the small number of tumours surviving in the DN-Maml1 mutant regions suggest that the lag time is small compared to the timescale of the experiment. It may also be the case that a small proportion of tumours are able to survive despite the competition with DN-Maml1 clones (see **Section 5** below).

4. Reducing competitive imbalance

Now that we have shown that the induction of a highly fit mutant following DEN treatment can eliminate tumours, we asked whether mutant clones already present in the DEN-treated tissue are able to remove tumours too. Fit clones present in the normal epithelium might be able to out-compete the tumours, eliminating them from the tissue (**Extended Data Figs. 9d-f**). By removing the competitive advantage of those clones, we can examine the impact they are having on tumour survival.

We assume there is a type of highly fit mutant clone, *N*, in the DEN-treated tissue that is able to remove tumours. As in the section above (Equation 2), we assume that the removal of clones

by mutant clones *N* is independent of other causes of tumour loss. Tumour survival probability is given by

$$p_{surv}^{ctl}(t) = p_{other}(t)\, p_N^{ctl}(t) \tag{8}$$

where, similar to above, $p_{other}(t)$ is the survival probability of a tumour based on all sources of tumour loss other than elimination by *N* clones, and $p_N^{ctl}(t)$ is the survival probability of a tumour assuming that the mutant *N* is the only cause of tumour loss. We assume that, without intervention, *N* clones spread progressively throughout the tissue and outcompete tumours they encounter, so $p_N^{ctl}(t_2) < p_N^{ctl}(t_1)$ for $t_2 > t_1$.

If we can raise the fitness of the surrounding tissue and tumours to a similar level as *N* clones, this would both prevent the spread of the *N* clones across the tissue (reducing the number of tumours directly competing with *N* clones) and reduce the elimination of tumours that are already adjacent to *N* clones, as they will now be competing neutrally (**Extended Data Fig. 10c**). Assuming that we have an intervention that completely levels the fitness of *N* clones with the rest of the tissue and tumours, the loss of tumours due to *N* clones will cease during that period, i.e. if the intervention starts at time t_1 and lasts until t_2, $p_N^{int}(t_2) = p_N^{int}(t_1) = p_N^{ctl}(t_1) > p_N^{ctl}(t_2)$ and

$$p_{surv}^{int}(t_2) = p_{other}(t_2)\, p_N^{int}(t_2) \;>\; p_{other}(t_2)\, p_N^{ctl}(t_2) = p_{surv}^{ctl}(t_2)$$

where, for the experiment in which the intervention is applied, $p_{surv}^{int}(t)$ is the overall tumour survival probability and $p_N^{int}(t)$ is the tumour survival probability related to the mutant *N*. Therefore, if we can remove or reduce the competitive imbalance between a mutant that can remove tumours and the rest of the tissue (including the tumours), then we should see an increase in surviving tumours compared to control experiments (**Extended Data Fig. 10d**).

Notch1 mutant clones dominate competition in the normal oesophageal epithelium[13], even at early time points (**Fig. 2g-i and Extended Data Figs. 7f-h**), and therefore is a good candidate mutation to test this prediction. The Notch inhibitor Dibenzazepine (DBZ) prevents Notch signalling and would affect all cells in the tissue, effectively raising the fitness of all Notch wild type clones and tumours to the level of the Notch mutant clones[11]. The competitive advantage of Notch mutants is thus removed during the DBZ intervention. Furthermore, by raising all cells to a high background level of fitness, this may also reduce the relative fitness advantage conveyed by mutations which work independently of the Notch pathway[49].

We indeed saw the predicted increase in surviving tumours when DBZ is administered between 10 days (t_1) and 24 days (t_2) after DEN treatment (**Extended Data Figs. 10e-g**). This suggests that competition from clones in the surrounding tissue is removing a substantial proportion of the tumours lost in the first few weeks following DEN treatment.

5. Selection pressure on tumours from competition with surrounding clones in the normal epithelium

There are genetic differences between the tumours sequenced 10 days and 1 year after DEN treatment, as shown by the dN/dS ratios (**Fig. 2c**) and the proportion of tumours mutant for each selected gene (**Extended Data Fig. 4j**). This genetic change over time could be consistent with ongoing selection of mutant subclones within tumours[24]. However, the elimination of early neoplasms by mutant clones in the surrounding tissue could also act as a selective pressure on tumours.

As described in **Section 4** above, the competitive fitness of a tumour compared to the surrounding clones in the normal tissue will affect the survival prospects of the tumour (**Fig. 3a**). If certain tumour genotypes have a competitive fitness comparable to or higher than the fittest mutant clones in the surrounding tissue, they would be more likely to survive. For example, *Notch1* is the dominant mutant gene in normal tissue, occupying almost the entire tissue 12 months after DEN treatment[13]. We might expect that, similar to the *Notch1* mutant clones in the normal tissue, tumours which are also *Notch1* mutant would be able to resist displacement by the mutant clones in the surrounding tissue. Consistent with this, we saw an increase in the proportion of *Notch1* mutant tumours from the 10-day to the 1-year time point (**Extended Data Fig. 4j**). We also see a large increase in the proportion of *Atp2a2* mutant tumours (**Extended Data Fig. 4j**), suggesting that these too may be able to resist displacement by clones in the surrounding tissue.

To explore this hypothesis, we expanded the model to include two tumour phenotypes: sensitive and resistant. Sensitive tumours can be removed by clones in the surrounding tissue, while resistant tumours cannot. We expect that the mutation rate is low following the cessation of DEN treatment (very few mutations spontaneously occur in untreated aged mice[13]), and therefore assume that, post-DEN treatment, sensitive tumours do not evolve resistance through mutation. The probability of tumour survival is then given by

$$p_{surv}(t) = S(0)\, p_{other}(t)\, p_N(t) + R(0)\, p_{other}(t) \tag{9}$$

where $S(0)$ and $R(0)$ are the proportions of tumours at day 0 following DEN treatment which are sensitive and resistant respectively.

The proportion of surviving tumours which are resistant to displacement by mutant clones, $R(t)$, is given by

$$R(t) = \frac{R(0)}{R(0) + S(0)p_N(t)} \tag{10}$$

Assuming that the number of sensitive and resistant tumours are non-zero, and that, as we assumed earlier, $p_N(t)$ is a decreasing function of time, then the proportion of surviving tumours which are resistant will increase over time.

The increasing proportion of the surviving tumours which are *Notch1* and/or *Atp2a2* mutant is consistent with the hypothesis that these mutations may increase the tumour's ability to resist displacement by clones in the surrounding tissue. The presence of a subset of tumours which are able to resist displacement by clones also could explain why a small fraction of tumours are able to survive over 1 year after DEN treatment (**Fig. 1d**) when the surrounding normal tissue is almost entirely populated by highly fit mutant clones[13].

6. Numerical model example

So far, we have mostly defined properties of the tumour survival probabilities rather than given specific equations for their values. This has allowed us to make testable predictions without requiring the details of the clonal or tumour dynamics to be defined.

Here we substitute feasible functions into Equation 9 to construct a numerical expression of the model. The functions are intended to be simple and introduce only a small number of model parameters, and the purpose here is to illustrate the concepts described in the previous sections rather than accurately and verifiably model the data.

Firstly, we need to define p_{other}, the non-clone related probability of tumour survival. As we have shown that apoptosis, abnormal proliferation of tumour cells, and the immune system are not contributing to tumour loss (**Extended Data Fig. 6**), we assume that p_{other} is simply the survival probability based on drift (Equation 1). Secondly, we need to define $p_N(t)$, the probability of tumour survival based on highly fit mutant clones in the surrounding tissue. Following the assumptions in **Sections 4 and 5**, we assume that there is a single mutant population *N* capable of removing tumours and that *N* mutant clones remove tumours as soon as they occupy the location of the tumour in the tissue. Our starting time occurs after the end of DEN treatment, and so much of the tissue may already be occupied by the *N* clones. Therefore, we modify Equation 3 to account for the sensitive tumours existing in the remaining non-*N* proportion of the tissue.

$$p_N(t) = \frac{1 - N(t)}{1 - N(0)} \tag{11}$$

This still leaves us having to define $N(t)$, the growth pattern of the mutant clones. Growth of mutant clones in oesophageal epithelium have previously been modelled using branching processes[14], but these don't consider competition between clones and limitations of the tissue size[13,25]. Cellular automaton simulations have also been used to model clones in this tissue[13], but this does not allow for easy integration with the mathematical formulation. Instead, we used

the logistic equation, which captures the key features of clonal spread: fast growth at early time points when mutant cells are mostly competing with surrounding wild type cells, slower growth at late time points when the tissue is already largely mutant, and an upper bound on total mutant spread[13]. Therefore,

$$N(t) = \frac{1}{1 + \left(\frac{1 - x_0}{x_0}\right) e^{-kt}} \tag{12}$$

where x_0 is the initial proportion of tissue covered by *N* mutant clones and k is the clone growth rate. To represent the experiment in which the DBZ Notch inhibitor is applied between 10 and 24 days after DEN, we can define

$$N_{DBZ}(t) = \begin{cases} N(t) & t < 10 \\ N(10) & 10 \leq t \leq 24 \end{cases} \tag{13}$$

The full model can then be constructed by substituting these expressions into equation 9.

We have 7 independent parameters: d, Δ, n, x_0, k, $S(0)$, and the initial tumour density (**Supplementary Table 11**). We used Approximate Bayesian Computation (ABC) to find the parameter combinations for which the model most closely matched the mean tumour density from 10 days to 18 months after DEN treatment and the mean tumour density in CTL and DBZ experiments at 24 days after DEN treatment (see **Methods**). The parameters were constrained as listed in **Supplementary Table 11**. The results are shown in **Extended Data Figs. 10h-i**. The median acceptable parameters found were (95% credible interval lower bound, upper bound) d=0.29/day (0.17, 0.35), Δ=0.020 (0.007, 0.047), n=1.4 (1.0, 2.5), x_0=0.80 (0.42, 0.99), k=0.032 (0.019, 0.040), $S(0)$=0.64 (0.44, 0.82) and initial tumour density=4.2/mm^2 (3.4, 5.4), although, given the simplifications and approximations used in this numerical example of the model, they should not be interpreted as estimates of their biological counterparts.

The numerical example of the modelling principles demonstrates how the elimination of (a subset of) tumours by mutant clones in the surrounding normal tissue leads to the experimental observations of decreasing tumour numbers following DEN treatment, higher tumour survival when competitive imbalance is removed (DBZ experiment), and a selection pressure on tumour genotype

7. Summary

Together, the experimental data and modelling indicate that tumours in the DEN-treated oesophageal epithelium are eliminated by mutant clones in the surrounding normal tissue. The data also suggests that the tumour genotype influences the chance of a tumour surviving, possibly by allowing the tumours to better compete with mutant clones in the surrounding tissue.

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