

# Supplementary Material to: Growth rate-driven modelling suggests that phenotypic adaptation drives drug resistance in BRAFV600E-mutant melanoma

Sara Hamis<sup>1</sup>\*, Alexander P Browning<sup>2</sup>, Adrienne L Jenner<sup>3</sup>, Chiara Villa<sup>4,5</sup>, Philip K Maini<sup>2</sup>, Tyler Cassidy<sup>6</sup>.

**1** Department of Information Technology, Uppsala University, Uppsala, Sweden.

**2** Mathematical Institute, University of Oxford, Oxford, United Kingdom.

**3** School of Mathematical Sciences, Queensland University of Technology, Brisbane, Australia.

**4** Sorbonne Université, CNRS, Université de Paris, Inria, Laboratoire Jacques-Louis Lions UMR, Paris, France.

**5** Université Paris-Saclay, Inria, Centre Inria de Saclay, 91120, Palaiseau, France.

**6** School of Mathematics, University of Leeds, Leeds, United Kingdom.

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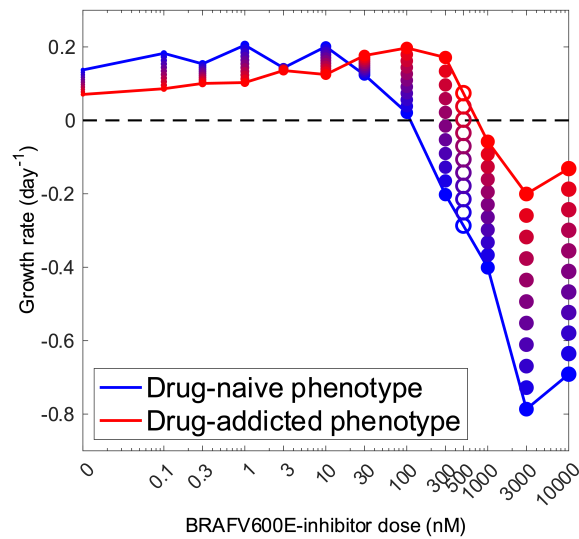
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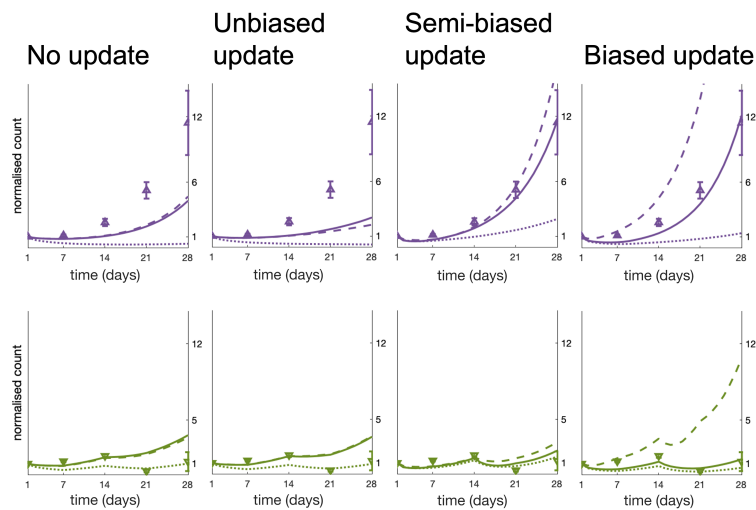
## SM1: Parameter fitting and complementary simulation results

In Figure 2d of the main manuscript, the treatment response models include four parameters that are fitted to match data. These are:  $FM(0, 500 \text{ nM})$ ,  $FM(1, 500 \text{ nM})$ ,  $\eta_{on}$ ,  $\eta_{off}$ . As an alternative approach, one could consider fixing  $FM(0, 500 \text{ nM})$ ,  $FM(1, 500 \text{ nM})$  via log-linear interpolation (the open circles in Supplementary Figure 1), and thereafter fitting only  $\eta_{on}$ ,  $\eta_{off}$ . However, with this approach, we were not able to find model parameters that yield simulations which simultaneously match both continuous and intermittent treatment responses. This result is shown in Supplementary Figure 2, which also demonstrates that for all update models, save the biased one, the 300 nM model and the 500 nM model with four free parameters result in similar and often overlapping simulated treatment dynamics.

In Supplementary Figures 3 to 6 we have reconstructed Figure 2d (with four free parameters) for different values of  $n$ , i.e., the number of intermittent phenotypes between the extrema  $x = 0$  and  $x = 1$ , and  $\rho$ , i.e., the probability that a cell accepts a proposed phenotype update. The results show that the directed (semi-biased and biased) update strategies better capture the cell count dynamics in response to treatments than do the non-directed (no update and unbiased) update strategies for tried values of  $n > 0$ , given the fitness matrix with linearly interpolated growth rates between the fitted extrema  $FM(0, 500 \text{ nM})$ ,  $FM(1, 500 \text{ nM})$ .



Supplementary Figure 1: 500 nM growth rates (open circles) can be estimated through log-linear interpolation between 300 nM and 1000 nM growth rates.



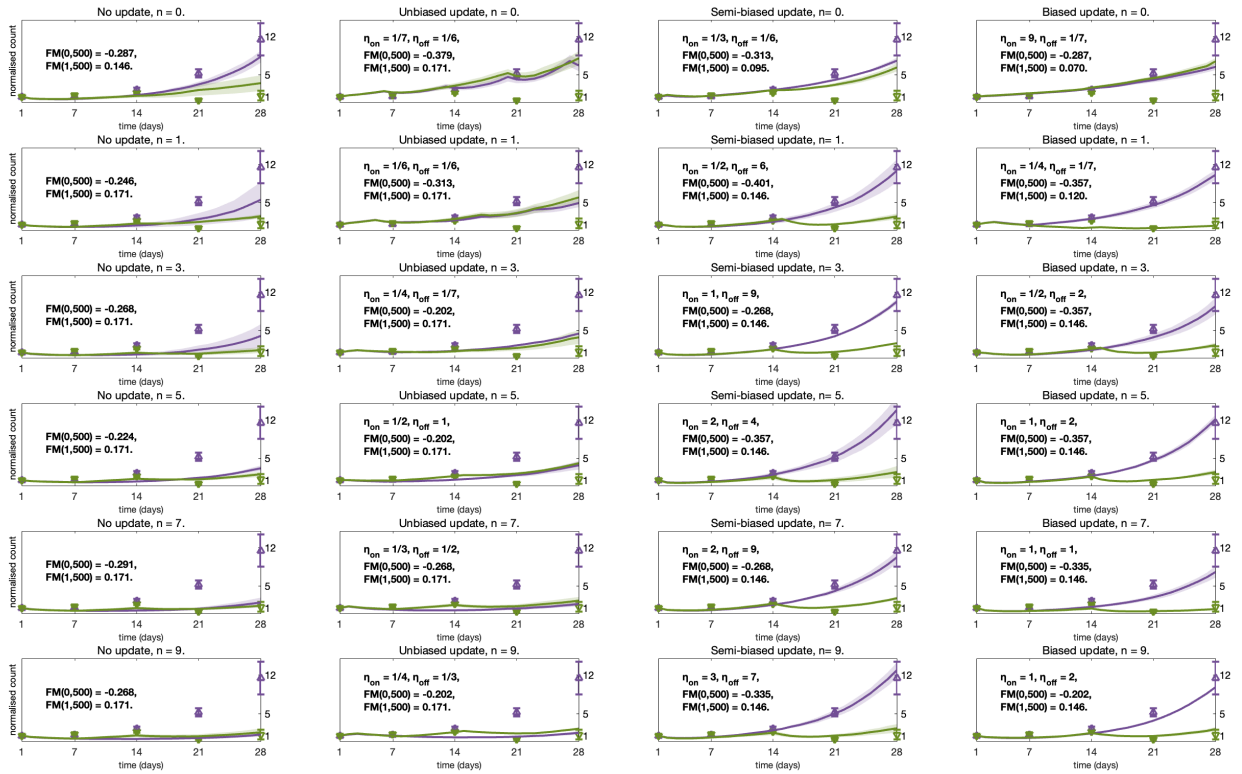
#### Continuous treatment

- ▲ *In vitro* data.
- Simulated responses to 300 nM with  $\eta_{on} = \eta_{off} = 2$ .
- Simulated responses to 500 nM with fitted FM(0,500), FM(1,500),  $\eta_{on}$ ,  $\eta_{off}$ .
- ⋯ Simulated responses to 500 nM with interpolated FM(0,500), FM(1,500) and fitted  $\eta_{on}$ ,  $\eta_{off}$ .

#### Intermittent treatment

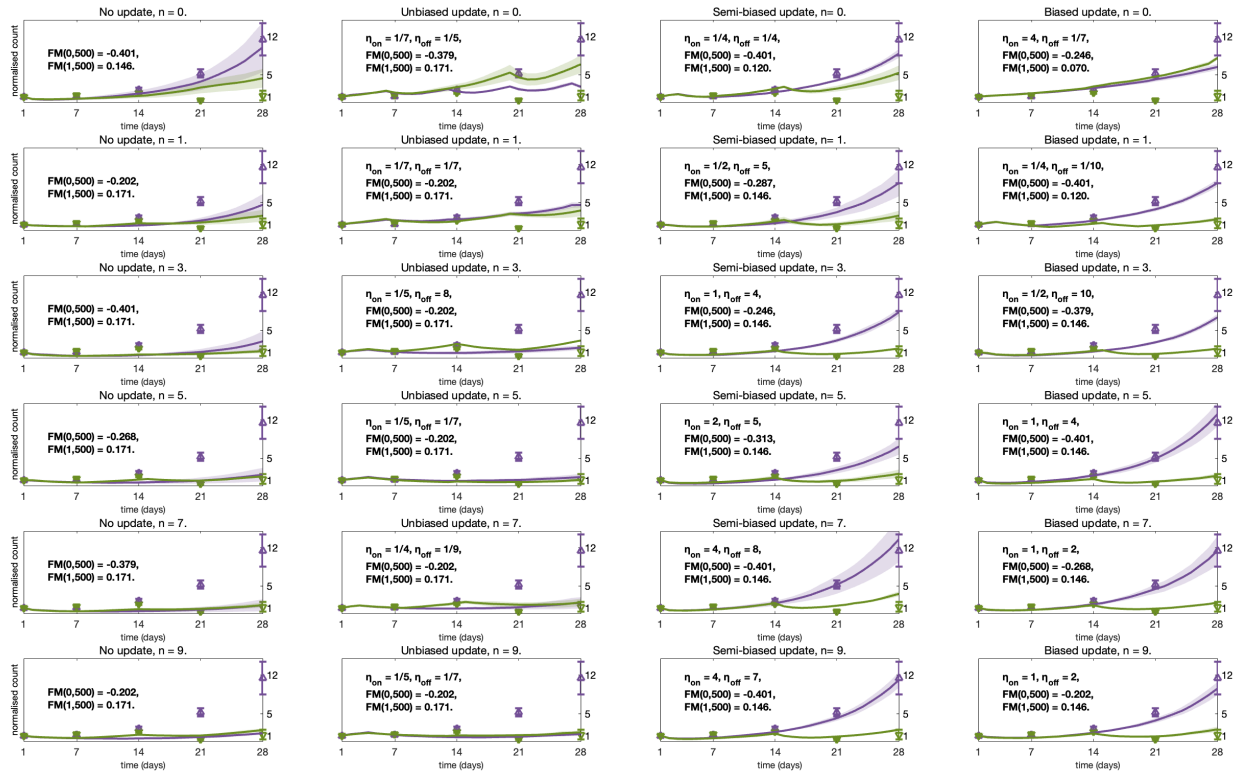
- ▼ *In vitro* data.
- Simulated responses to 300 nM with  $\eta_{on} = \eta_{off} = 2$ .
- Simulated responses to 500 nM with fitted FM(0,500), FM(1,500),  $\eta_{on}$ ,  $\eta_{off}$ .
- ⋯ Simulated responses to 500 nM with interpolated FM(0,500), FM(1,500) and fitted  $\eta_{on}$ ,  $\eta_{off}$ .

Supplementary Figure 2: Simulated responses to continuous (top) and intermittent (bottom) treatments are shown for different phenotype update strategies (left-to-right) and three different model parameter combinations (see legend). *In vitro* data (triangles) are also shown.



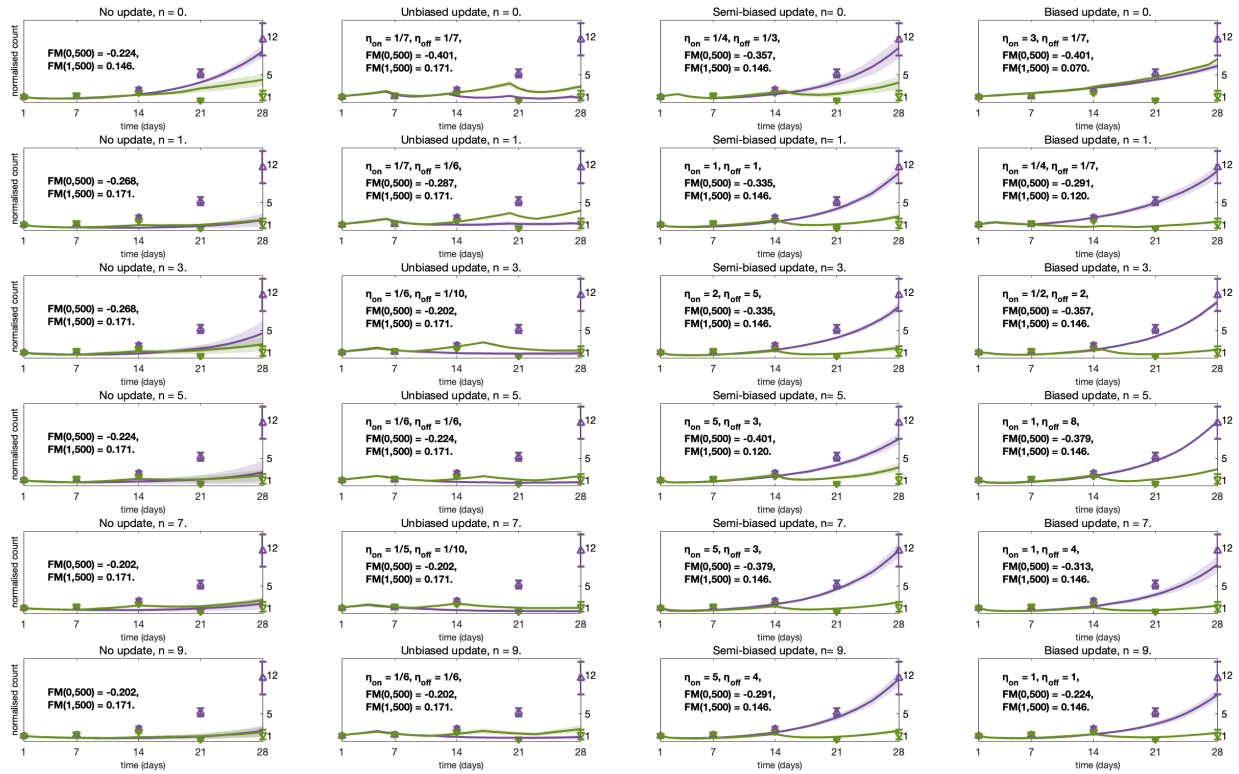
Supplementary Figure 3: **Simulated treatment responses for  $\rho = 1$** . Plots are shown for different phenotype update strategies (left to right), and for varying numbers of intermediate phenotypes  $n$  between the extrema  $x = 0$  and  $x = 1$ . The number of phenotypes  $n$  increases as we go from higher to lower rows. In each plot, the model parameters  $\eta_{on}$ ,  $\eta_{off}$ ,  $FM(x = 0, d = 500)$ ,  $FM(x = 1, d = 500)$  that simultaneously minimise the root mean squared error between data and simulations for both continuous (purple) and intermittent (green) treatment responses to 500 nM doses are used.

0.75



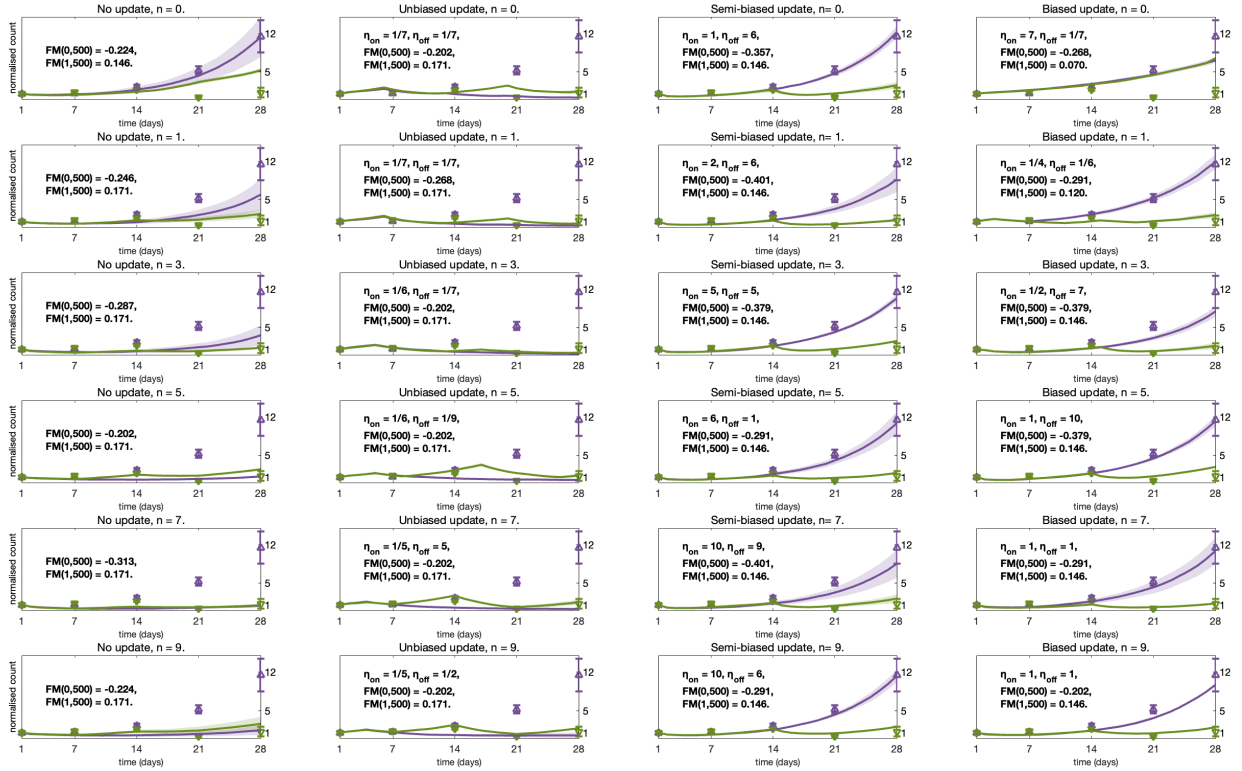
Supplementary Figure 4: **Simulated treatment responses for  $\rho = 0.75$ .** Plots are shown for different phenotype update strategies (left to right), and for varying numbers of intermediate phenotypes  $n$  between the extrema  $x = 0$  and  $x = 1$ . The number of phenotypes  $n$  increases as we go from higher to lower rows. In each plot, the model parameters  $\eta_{on}$ ,  $\eta_{off}$ ,  $FM(x = 0, d = 500)$ ,  $FM(x = 1, d = 500)$  that simultaneously minimise the root mean squared error between data and simulations for both continuous (purple) and intermittent (green) treatment responses to 500 nM doses are used.

0.5



Supplementary Figure 5: **Simulated treatment responses for  $\rho = 0.5$ .** Plots are shown for different phenotype update strategies (left to right), and for varying numbers of intermediate phenotypes  $n$  between the extrema  $x = 0$  and  $x = 1$ . The number of phenotypes  $n$  increases as we go from higher to lower rows. In each plot, the model parameters  $\eta_{on}$ ,  $\eta_{off}$ ,  $FM(x = 0, d = 500)$ ,  $FM(x = 1, d = 500)$  that simultaneously minimise the root mean squared error between data and simulations for both continuous (purple) and intermittent (green) treatment responses to 500 nM doses are used.

0.25



Supplementary Figure 6: **Simulated treatment responses for  $\rho = 0.25$ .** Plots are shown for different phenotype update strategies (left to right), and for varying numbers of intermediate phenotypes  $n$  between the extrema  $x = 0$  and  $x = 1$ . The number of phenotypes  $n$  increases as we go from higher to lower rows. In each plot, the model parameters  $\eta_{on}$ ,  $\eta_{off}$ ,  $FM(x = 0, d = 500)$ ,  $FM(x = 1, d = 500)$  that simultaneously minimise the root mean squared error between data and simulations for both continuous (purple) and intermittent (green) treatment responses to 500 nM doses are used.

## SM2: Expressions for effective growth rates under intermittent treatments

A Wolfram Mathematica notebook for calculating long-term effective growth rates for intermittent treatment schedules (Eq. 6 in the main article) are available on the GitHub repository [https://github.com/SJHamis/phenotype\\_adaptation](https://github.com/SJHamis/phenotype_adaptation). A printed version of the notebook is provided on the next few pages.

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# Growth-rate driven modelling study

Supplementary material to *Hamis, Browning, Jenner, Villa, Maini, Cassidy (2025)*.

In this Notebook we calculate

$$\lambda_{\text{eff}} = \frac{1}{\tau} \left( \int_0^{t_{\text{on}}} \text{FM}(x(t), d) dt + \int_{t_{\text{on}}}^{t_{\text{off}}} \text{FM}(x(t), 0) dt \right)$$

for different choices of  $t_{\text{on}}$  (the duration that the drug is *on*) and  $t_{\text{off}}$  (the duration that the drug is *off*).

## Define growth rates as functions of the phenotype state $x$ :

In the notebook,  $\text{FM}_{xd}$  denotes  $\text{FM}(x,d)$  where  
 $x=0$  is the least drug-adapted phenotype state,  
 $x=1$  is the most drug adapted phenotype state,  
 $d=0$  means “drug is off”,  
 $d=D$  means “drug is on” at a selected dose  $D$ .

```
In[1]:= λ0[x_] := FM00 + (FM10 - FM00) x (* no drug *)
λ1[x_] := FM0D + (FM1D - FM0D) x (* drug *)
```

## State assumptions:

```
In[3]:= $Assumptions = ω > 0;
```

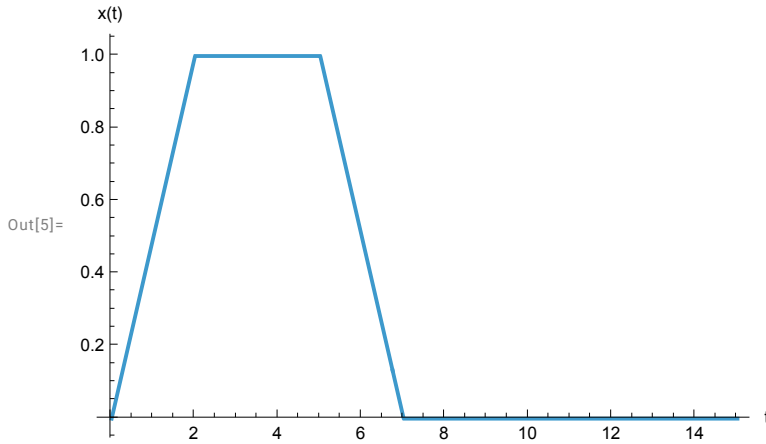
Case 1:  $t_{\text{on}}, t_{\text{off}} > \frac{1}{\omega}$ .

**Drug on:** Phenotypes start at  $x=0$ , reach  $x=1$  during climbing period of length  $1/\omega$ , and then stay at  $x=1$  until time  $t_{\text{on}}$ .

**Drug off:** Cells reach  $x=0$  during a descending period of length  $1/\omega$ , and then stay at  $x=0$  until time  $t_{\text{on}} + t_{\text{off}}$ .

A plot is shown below for (arbitrarily selected) values of  $t_{\text{on}}, t_{\text{off}}, \omega$  such that Case 1 holds.

```
In[4]:= x[t_] := Piecewise[{{ω t, t < 1/ω}, {1, 1/ω ≤ t ≤ ton},
  {1 - ω (t - ton), ton < t < ton + 1/ω}, {0, t ≥ ton + 1/ω}}];
Plot[x[t] /. {ω → 0.5, ton → 5, toff → 10}, {t, 0, 15}, AxesLabel → {"t", "x(t)"}]
```



We now symbolically calculate and simplify the expression for the Case 1 integral using  $\Delta FM(d) = FM(1, d) - FM(0, d)$ .

```
In[6]:= C1 = 1/τ FullSimplify[
  Integrate[λ1[ω t], {t, 0, 1/ω}] +
  Integrate[λ1[1], {t, 1/ω, ton}] +
  Integrate[λ0[1 - ω (t - ton)], {t, ton, ton + 1/ω}] +
  Integrate[λ0[0], {t, ton + 1/ω, ton + toff}]
] /. {FM10 - FM00 → ΔFM0} /. {FM0D - FM1D → -ΔFMD}
Out[6]= (FM00 toff + FM1D ton + (ΔFM0 - ΔFMD) / (2 ω)) / τ
```

Now customised, numerical values for  $t_{on}$ ,  $t_{off}$ ,  $\omega$ , can be inserted into the expression for C1 to obtain  $\lambda_{eff}$ .

```
In[7]:= C1num = C1 /. {ΔFM0 → FM10 - FM00, ΔFMD → FM1D - FM0D, τ → ton + toff};
(*Writing C1 in long format*)
C1num /. {FM0D → -0.2866, FM1D → 0.0741, FM00 → 0.1374, FM10 → 0.07067,
  ω → 0.5, ton → 5, toff → 10} (*As used in the above plot*)
Out[8]= 0.0878047
```

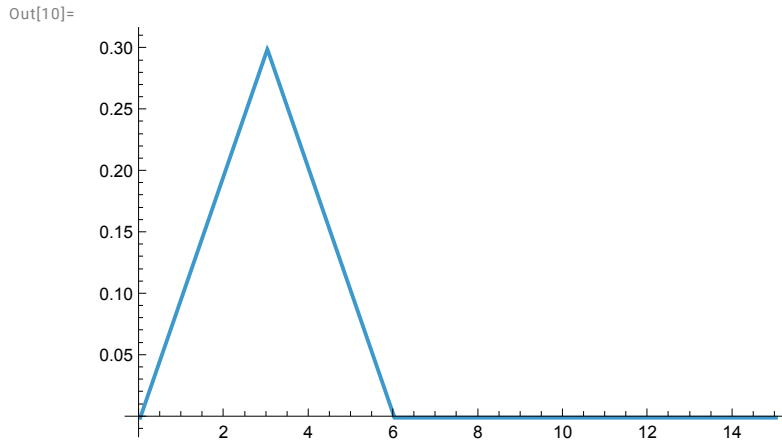
**Case 2:**  $t_{on} < t_{off} < \frac{1}{\omega}$ .

**Drug on:** Phenotypes start at  $x=0$ , and then reach  $x=\omega t$  during climbing period of length  $t_{on}$ .

**Drug off:** Cells reach  $x=0$  during a descending period of length  $t_{on}$ , and then stay at  $x=0$  until time  $t_{on} + t_{off}$ .

A plot is shown below for (arbitrarily selected) values of  $t_{on}$ ,  $t_{off}$ ,  $\omega$  such that Case 2 holds.

```
In[9]:= x[t_] := Piecewise[
  {{\omega t, t < ton}, {\omega ton - \omega (t - ton), ton \le t < 2 ton}, {0, 2 ton \le t \le ton + toff}}]
Plot[x[t] /. {\omega \to 0.1, ton \to 3, toff \to 10}, {t, 0, 15}]
```



We now symbolically calculate and simplify the expression for the Case 2 integral using  $\Delta FM(d) = FM(1, d) - FM(0, d)$ .

```
In[11]:= C2 = 1/\tau FullSimplify[
  Integrate[\lambda1[\omega t], {t, 0, ton}] +
  Integrate[\lambda0[\omega ton - \omega (t - ton)], {t, ton, 2 ton}] +
  Integrate[\lambda0[0], {t, 2 ton, ton + toff}]
] /. {FM10 - FM00 \to \Delta FM0} /. {-FM0D + FM1D \to \Delta FM1}
```

Out[11]=

$$\frac{FM00 \text{ toff} + FM0D \text{ ton} + \frac{1}{2} \text{ ton}^2 (\Delta FM0 + \Delta FM1) \omega}{\tau}$$

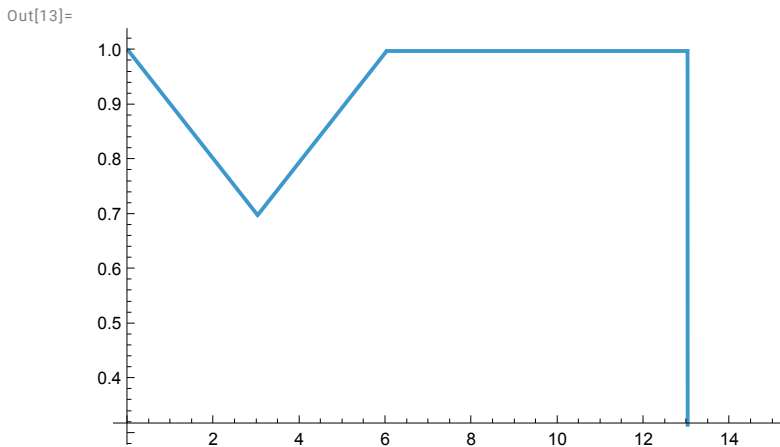
**Case 3:**  $t_{off} < t_{on} < \frac{1}{\omega}$ .

**Drug off:** Phenotypes start at  $x=1$ , and then reach  $x=\omega t$  during descending period of length  $t_{off}$ .

**Drug on:** Phenotypes reach  $x=1$  during climbing period of length  $t_{off}$ , and then stay at  $x=1$  until time  $t_{on} + t_{off}$ .

A plot is shown below for (arbitrarily selected) values of  $t_{on}$ ,  $t_{off}$ ,  $\omega$  such that Case 2 holds.

```
In[12]:= x[t_] := Piecewise[{{1 - ω t, t < toff},
  {1 - ω toff + ω (t - toff), toff ≤ t < 2 toff}, {1, 2 toff ≤ t ≤ ton + toff}}]
Plot[x[t] /. {ω → 0.1, ton → 10, toff → 3}, {t, 0, 15}]
```



```
In[14]:= C3 = 1/T FullSimplify[
  Integrate[λ0[1 - ω t], {t, 0, toff}] +
  Integrate[λ1[1 - ω toff + ω (t - toff)], {t, toff, 2 toff}] +
  Integrate[λ1[1], {t, 2 toff, ton + toff}]
] /. {FM0D - FM1D → -ΔFMD} /. {FM00 - FM10 → -ΔFM0}
```

Out[14]=

$$\frac{FM10 \text{ toff} + FM1D \text{ ton} + \frac{1}{2} \text{ toff}^2 (-\Delta FM0 - \Delta FMD) \omega}{T}$$

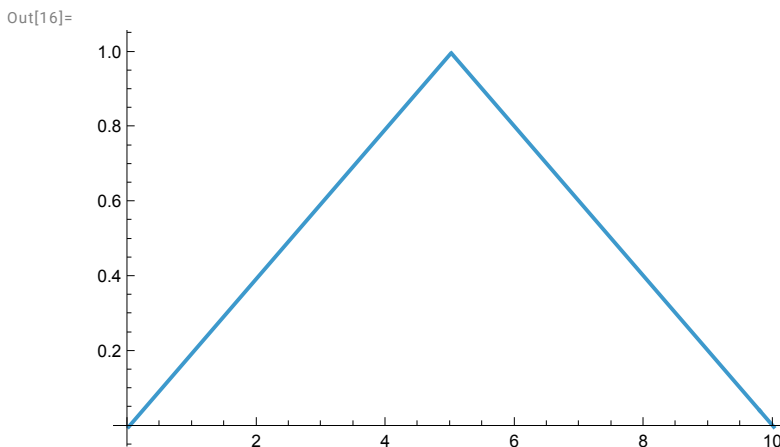
Case 4:  $t_{on} = t_{off} = \frac{T}{2} = \frac{1}{\omega}$ .

**Drug on:** Phenotypes start at  $x=0$ , and then reach  $x=1$  during climbing period of length  $\frac{T}{2}$ .

**Drug off:** Cells reach  $x=0$  during a descending period of length  $\frac{T}{2}$ .

A plot is shown below for (arbitrarily selected) values of  $t_{on} = t_{off}$  such that Case 2 holds.

```
In[15]:= x[t_] := Piecewise[{{ω t, t < ton}, {1 - ω (t - ton), ton ≤ t < 2 ton}}]
Plot[x[t] /. {ω → 1/5, ton → 5}, {t, 0, 10}]
```



We now symbolically calculate and simplify the expression for the Case 4 integral.

```
In[17]:= C4 =  $\frac{1}{2 * \text{ton}}$  FullSimplify[
  Integrate[\(\lambda 1[\omega t]\), {t, 0, ton}] +
  Integrate[\(\lambda 0[1 - \omega (t - (\text{ton}))]\), {t, ton, 2 * ton}]
] /. {ton * \(\omega \rightarrow 1\)} /. {-FM0D - FM10 + 2 (FM0D + FM10) \(\rightarrow FM0D + FM10\)}
```

```
Out[17]=  $\frac{1}{4} (FM00 + FM0D + FM10 + FM1D)$ 
```

We now consider Case 5 and 6 where  $t_{\text{on}} = t_{\text{off}} = T$ .

Case 5:  $T < \frac{1}{\omega}$ .

Case 6:  $T > \frac{1}{\omega}$ ,

```
In[18]:= C5 =  $\frac{1}{2 T}$  FullSimplify[
  Integrate[\(\lambda 1[\omega t]\), {t, 0, T}] +
  Integrate[\(\lambda 0[\omega T - \omega (t - T)]\), {t, T, 2 T}]
] /. {-FM0D + FM1D \(\rightarrow \Delta FMD\)} /. {-FM00 + FM10 \(\rightarrow \Delta FM0\)}
```

```
Out[18]=  $\frac{1}{4} (2 (FM00 + FM0D) + T (\Delta FM0 + \Delta FMD) \omega)$ 
```

```
In[19]:= C6 =  $\frac{1}{2 T}$  FullSimplify[
  Integrate[\(\lambda 1[\omega t]\), {t, 0, 1/\(\omega\)}] +
  Integrate[\(\lambda 1[1]\), {t, 1/\(\omega\), T}] +
  Integrate[\(\lambda 0[1 - \omega (t - T)]\), {t, T, T + 1/\(\omega\)}] +
  Integrate[\(\lambda 0[0]\), {t, T + 1/\(\omega\), 2 T}]
] /. {FM0D - FM1D \(\rightarrow -\Delta FMD\)} /. {-FM00 + FM10 \(\rightarrow \Delta FM0\)}
```

```
Out[19]= 
$$\frac{(FM00 + FM1D) T + \frac{\Delta FM0 - \Delta FMD}{2 \omega}}{2 T}$$

```

### SM3: Model parameters used in the main article results figures

The model parameters used to generate the simulation results shown in the main article are listed in Supplementary Table 1.

panel	$\eta_{on}$	$\eta_{off}$	FM(0,0 nM)	FM(1,0 nM)	FM(0,300 nM)	FM(1,300 nM)	FM(0,500 nM)	FM(1,500 nM)	initial condition
<b>Figure 2</b>									
c; 300 nM; no update	-	-	0.137 day <sup>-1</sup>	0.071 day <sup>-1</sup>	-0.202 day <sup>-1</sup>	0.171 day <sup>-1</sup>	-	-	Figure 4 (day 1)
c; 300 nM; all other panels	2	2	0.137 day <sup>-1</sup>	0.071 day <sup>-1</sup>	-0.202 day <sup>-1</sup>	0.171 day <sup>-1</sup>	-	-	Figure 4 (day 1)
c; 500 nM; no update	-	-	0.137 day <sup>-1</sup>	0.071 day <sup>-1</sup>	-	-	-0.202 day <sup>-1</sup>	0.171 day <sup>-1</sup>	uniform
c; 500 nM; unbiased	1	3	0.137 day <sup>-1</sup>	0.071 day <sup>-1</sup>	-	-	-0.335 day <sup>-1</sup>	0.146 day <sup>-1</sup>	uniform
c,e; 500 nM; semi-biased	3	4	0.137 day <sup>-1</sup>	0.071 day <sup>-1</sup>	1	3	-0.268 day <sup>-1</sup>	0.171 day <sup>-1</sup>	left-shifted half normal
c,e; 500 nM; biased	-	-	0.137 day <sup>-1</sup>	0.071 day <sup>-1</sup>	-	-	-0.202 day <sup>-1</sup>	0.171 day <sup>-1</sup>	left-shifted half normal
<b>Figure 3</b>									
b; 300 nM	$\eta$	$\eta$	0.137 day <sup>-1</sup>	0.071 day <sup>-1</sup>	-0.202 day <sup>-1</sup>	0.071 day <sup>-1</sup>	-	-	Figure 3a
e; 300 nM	$\eta$	$\eta$	0.137 day <sup>-1</sup>	0.071 day <sup>-1</sup>	-0.202 day <sup>-1</sup>	0.071 day <sup>-1</sup>	-	-	Figure 3d
b,c; 500 nM	$\eta$	$\eta$	0.137 day <sup>-1</sup>	0.071 day <sup>-1</sup>	-	-	-0.287 day <sup>-1</sup>	0.074 day <sup>-1</sup>	Figure 3a
e,f; 500 nM	$\eta$	$\eta$	0.137 day <sup>-1</sup>	0.071 day <sup>-1</sup>	-	-	-0.287 day <sup>-1</sup>	0.074 day <sup>-1</sup>	Figure 3d
<b>Figure 4</b>									
no update	-	-	0.137 day <sup>-1</sup>	0.071 day <sup>-1</sup>	-0.202 day <sup>-1</sup>	0.171 day <sup>-1</sup>	-	-	in top figure row
all other panels	2	2	0.137 day <sup>-1</sup>	0.071 day <sup>-1</sup>	-0.202 day <sup>-1</sup>	0.171 day <sup>-1</sup>	-	-	in top figure row

Supplementary Table 1: Model parameters used to in the simulation results presented in the main article. The initial phenotype distribution (initial condition) is described in the right-most column.