Tumor growth characteristics

Tumor growth rates are chosen to produce median 'potential tumor doubling time' (DT_{pot}) of 10 days (that is, cell doubling rate when not accounting for cell loss), with a cell loss factor (ϕ) of 80% so as to produces tumor volume doubling times (DT) of median 50 days, with 10% and 90% quantiles of ~36 days and ~83 days. This is achieved with each tumor's growth rate parameter drawn from a normal distribution with mean 0.069 $days^{-1}$, standard deviation 0.021 $days^{-1}$ (and an enforced minimum of 0.01 to prevent negative growth rates).

These parameters (potential tumor volume doubling time, actual tumor volume doubling time, cell loss factor) are chosen as being approximately representative of human carcinoma according to the following references:

Steel GG. Cell loss as a factor in the growth rate of human tumours. European journal of cancer 1967;3:381-7

Steel GG. Growth kinetics of tumours : cell population kinetics in relation to the growth and treatment of cancer. Oxford Eng.: Clarendon Press; 1977

Refsum SB, Berdal P. Cell loss in malignant tumours in man. European journal of cancer 1967;3:235-6.

Frindel E, Malaise E, Tubiana M. Cell proliferation kinetics in five human solid tumors. Cancer 1968;22:611-20.

Malaise EP, Chavaudra N, Tubiana M. The relationship between growth rate, labelling index and histological type of human solid tumours. European journal of cancer 1973;9:305-12.

Silvestrini R, Sanfilippo O, Tedesco G. Kinetics of human mammary carcinomas and their correlation with the cancer and the host characteristics. Cancer 1974;34:1252-8.

Kerr KM, Lamb D. Actual growth rate and tumour cell proliferation in human pulmonary neoplasms. British journal of cancer 1984;50:343-9.

Tubiana M. Tumor cell proliferation kinetics and tumor growth rate. Acta oncologica 1989;28:113-21.

Charbit A, Malaise EP, Tubiana, M. Relation between the pathological nature and the growth rate of human tumors. European journal of cancer 1971;7:307-15

For example:

Refsum & Berdal (1967) report epidermoid and salivary gland carcinomas with mean DT of 60 days, DT_{pot} of 2.4 days, and ϕ=96%

Silvestrini, Sanfilippo, and Tedesco (1974) report mammary carcinomas as having median DT from 85 to 105 days (depending on source study) in primary tumors, and 40 days in recurrent tumors. DT_{pot} is reported as ranging from ~8 to ~28 days, with slower rates in older patients.

Kerr & Lamb (1984) report pulmonary tumors having mean ϕ = 90% (ranging from 71% to 99%; excluding lower values of ϕ in secondary renal tumors), mean DT = 73 days (ranging from 20 days to 154 days), and mean DT_{pot} = 4.8 days (ranging from 2.2 days to 7.1 days).

Charbit, Malaise, and Tubiana (1971) report the following doubling times:

Pathology	Median DT (days)
Lung metastasis of embryonal tumors	25
Lymphomas	32
Malignant mesenchymal tumors	37
Lung metastasis of squamous cell carcinoma	56
Primary squamous cell carcinoma	80
Lung metastasis of adenocarcinoma	89

Primary adenocarcinoma	207

Malaise, Chavaudra, and Tubiana (1973) and Tubiana (1989) reports the following mean tumor kinetic parameters:

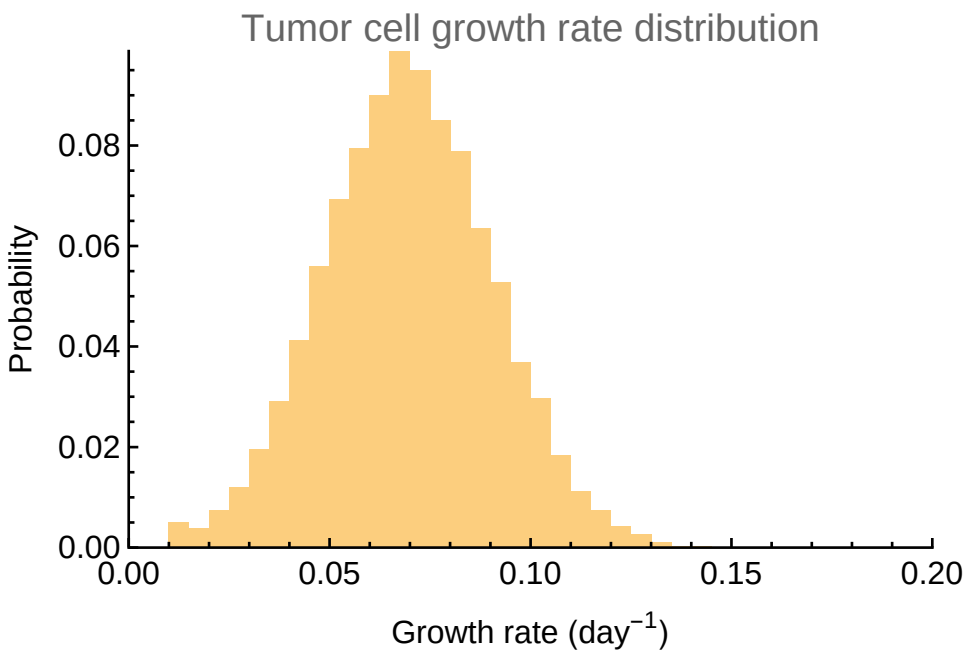
Histological type	DT (days)	ϕ (%)	DT_{pot} (days)
Embryonal tumors	27	93	2
Hematosarcoma	29	94	2
Malignant lymphomas	29	93	2
Mesenchymal sarcomas	41	68	13
Squamous cell carcinomas	58	89	6
Adenocarcinomas	83	71	24

Steel (1967) reports potential tumor doubling times:

Site of tumor	DT_{pot} (days)
Breast	43
Colon	10.4
Stomach	6.8
CNS	23.4
Melanoma	14.2
Lung and Larynx	3.1
Tongue	6.5
Uterus and Cervix	9.8
Lymphosarcoma, Burkitt Tumor	1.5
Miscellaneous sites	33

Distribution of growth rates

```
CellLossRate = 80 / 100;
DistributionOfGrowthRates =
  Table[Max[{1 / 100, RandomVariate[NormalDistribution[6.9 / 100, 2.1 / 100]]}], {10^4}];

Histogram[DistributionOfGrowthRates, {0, 0.2, 0.005}, "Probability", ChartStyle → EdgeForm[None],
 Frame → {{True, False}, {True, False}}, BaseStyle → {FontFamily → "Arial", FontSize → 12},
 FrameStyle → Directive[Black, Thickness[Medium]], PlotRangePadding → None,
 FrameLabel → {"Growth rate (day^-1)", "Probability"},
 PlotLabel → "Tumor cell growth rate distribution"]
```



Median DTpot and DT

```
Off[Solve::ifun]
(* solving for median potential tumor doubling time, DT_pot *)
MedianCellDoublingTime = Solve[Exp[Median[DistributionOfGrowthRates] * (1) * t] == 2]
(* solving for median actual tumor doubling time, DT *)
MedianTumorDoublingTime =
 Solve[Exp[Median[DistributionOfGrowthRates] * (1 - CellLossRate) * t] == 2]
```

```
{{t → 9.99373}}
```

```
{{t → 49.9686}}
```

10 % and 90 % quantiles of DTpot and DT

```
(* 10% and 90% quantile values for DT_pot *)
Quantile10CellDoublingTime = Solve[Exp[Quantile[DistributionOfGrowthRates, 0.1] * (1) * t] == 2]
Quantile90CellDoublingTime = Solve[Exp[Quantile[DistributionOfGrowthRates, 0.9] * (1) * t] == 2]


(* 10% and 90% quantile values for DT *)
Quantile10TumorDoublingTime =
 Solve[Exp[Quantile[DistributionOfGrowthRates, 0.1] * (1 - CellLossRate) * t] == 2]
Quantile90TumorDoublingTime =
 Solve[Exp[Quantile[DistributionOfGrowthRates, 0.9] * (1 - CellLossRate) * t] == 2]
```

```
{{t → 16.0715}}
```

```
{{t → 7.18467}}
```

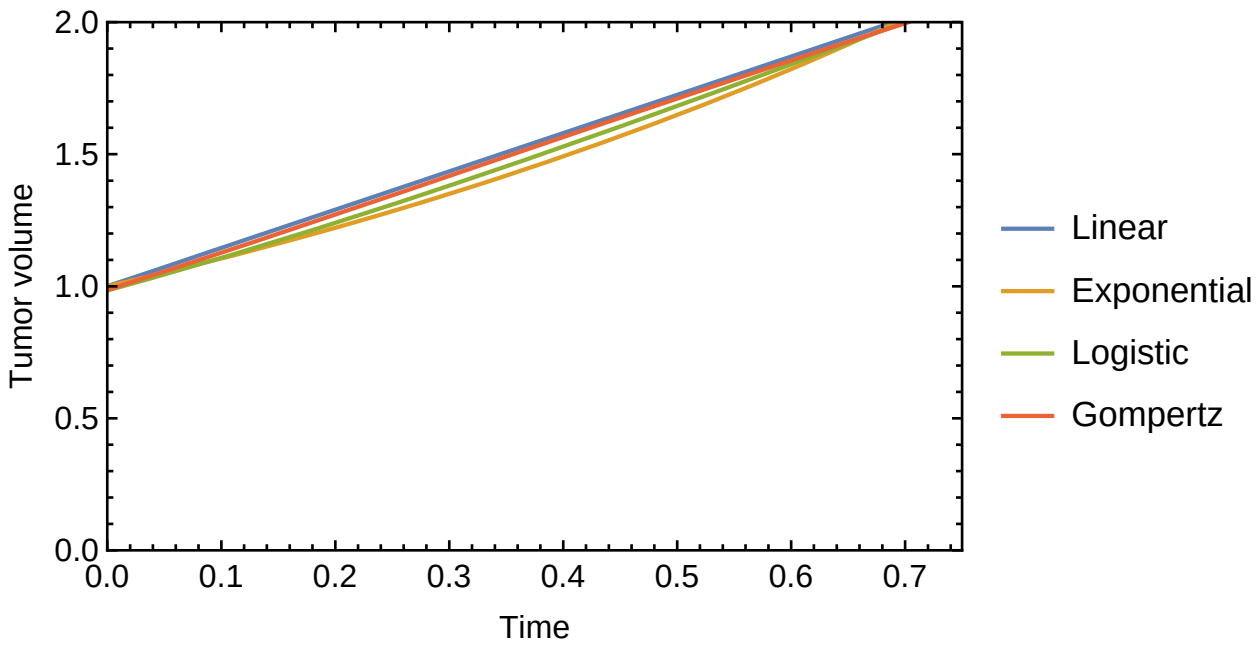
```
{{t → 80.3575}}
```

```
{{t → 35.9234}}
```

Note that the model output is time taken for tumor volume to double ('progression'), and over a 2-fold growth rate, there is little difference in the growth trajectories of exponential, logistic, gompertz, or even linear growth models (when calibrated to have same net doubling rate)

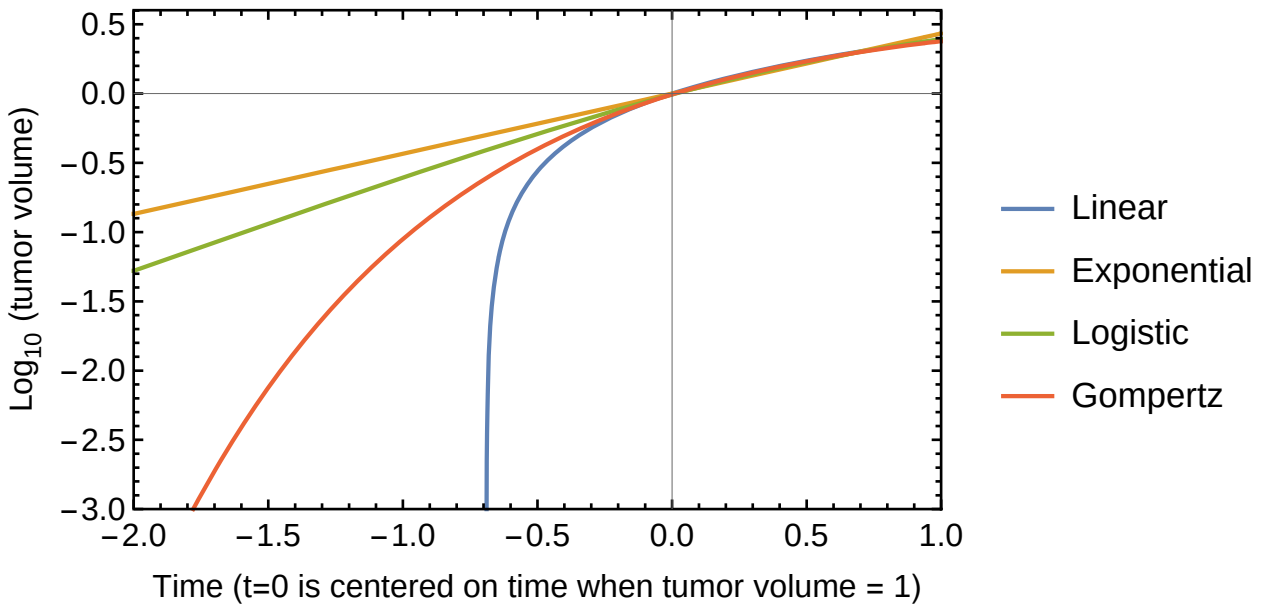
```
Exponential[t_] := Exp[k * t] /. {k → 1}

Linear[t_] := 1 + k * t /. {k → 1.45}

(* here, logistic and Gompertz growth models are calibrated to have a maximum
 population that is twice as large as the threshold for progression,
itself being twice the initial population size. When calibrated to have a larger
 maximum population, their difference from exponential growth model is yet smaller *)
Logistic[t_] := L / (1 + Exp[-k * (t - t0)]) /. {L → 4, k → 1.6, t0 → 0.7}

Gompertz[t_] := a * Exp[-b * Exp[-c * t]] /. {a → 4, b → 1.4, c → 1}
```

```
Plot[{Linear[t], Exponential[t], Logistic[t], Gompertz[t]}, {t, 0, 0.75}, PlotRange → {0, 2},
 PlotLegends → {"Linear", "Exponential", "Logistic", "Gompertz"}, Frame → True,
 FrameLabel → {"Time", "Tumor volume"}, PlotRangePadding → None,
 BaseStyle → {FontFamily → "Arial", FontSize → 12},
 FrameStyle → Directive[Black, Thickness[Medium]]]
```



There is greater divergence between growth models in the case of substantial tumor shrinkage:

```
Plot[{Log[10, Linear[t]], Log[10, Exponential[t]], Log[10, Logistic[t]], Log[10, Gompertz[t]]},
 {t, -2, 1}, PlotRange → {-3, Log[10, 4]},
 PlotLegends → {"Linear", "Exponential", "Logistic", "Gompertz",
   "Fastest feasible cell division rate"}, Frame → True,
 FrameLabel → {"Time (t=0 is centered on time when tumor volume = 1)", "Log10 (tumor volume)"},
 PlotRangePadding → None, BaseStyle → {FontFamily → "Arial", FontSize → 12},
 FrameStyle → Directive[Black, Thickness[Medium]]]
```



The effect of implementing 'Gompertzian' growth kinetics are explored at the very end of this document.

Modeling drug action

Drug inhibition of tumor growth is modeled as a simple ligand-receptor interaction, with relative growth rate of cells decreasing in proportion to target occupancy by drug.

Thus, relative growth = $\frac{1}{1+[\text{drug}]/K_I}$, where [drug] = drug concentration, and K_I= inhibitory constant, being the drug concentration that produces 50% inhibition.

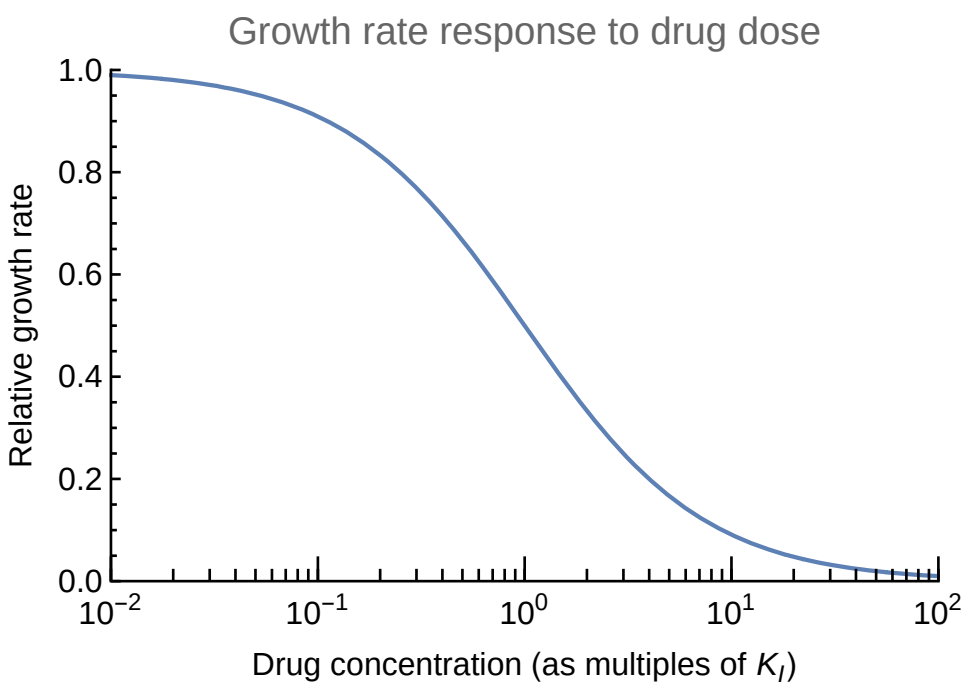
A Hill coefficient greater than 1 could also be modeled $\left(\frac{1}{1+([\text{drug}]/K_I)^{\text{Hill coefficient}}}\right)$, but here we take Hill coefficient = 1.

Note that in this drug inhibition model, [drug] and K_I only appear as their dimensionless ratio $[\text{drug}]/K_I$. Both [drug] and K_I have dimensions of concentration, and thus their units cancel out in the ratio. Therefore, any specific choice of units is not relevant to the model; only the ratio of drug concentration to inhibitory constant matters.

Most simply, drug treatment can be modeled as continuous, but this model can also be adjusted to describe time-dependent therapy (e.g. periodic cycles of a cytotoxic therapy).
Time-dependent treatment can be implementing using the ‘DrugTreatmentOverTime’ function - but in the present instance therapy is modeled as continuous.

```
(* function describing drug response over time. Most simply, therapy is continuous,
but this function can be used to describe time-
 dependent therapy (e.g. periodic cycles of treatment) *)
DrugTreatmentOverTime[t_, RelativeGrowthDuringTreatment_] := RelativeGrowthDuringTreatment
(* continuous therapy *)

(* response of growth to drug treatment - a simple Hill function,
hyperbolic in the case of Hill Coefficient NH=1. d = drug dose,
KI = drug sensitivity (IC50) *)
DrugInhibitionModel = 1 / (1 + (drug / KI)^NH);

(* same model as above,
but expressing drug concentration and KI as their logarithms. This makes for easier
  plotting on a log-scale of drug concentration *)
LogDrugInhibitionModel = 1 / (1 + (10^LogDrug / 10^LogKI)^NH);
```

```
(* custom axis ticks for log scale *)
logframeticks = Join[Table[{x, "10"^ToString[x], {0.03, 0}}, {x, -10, 10}],
   Flatten[Table[Table[{Log[10, x], "", {0.015, 0}}, {x, 1 * 10^y, 9 * 10^y, 1 * 10^y}], {y, -10, 10, 1}],
    1]];

Plot[(LogDrugInhibitionModel /. {LogKI → 0, NH → 1}), {LogDrug, -2, 2}, PlotRange → {0, 1},
 Frame → {{True, False}, {True, False}}, Axes → False,
 BaseStyle → {FontFamily → "Arial", FontSize → 12},
 FrameStyle → Directive[Black, Thickness[Medium]], PlotRangePadding → None,
 FrameLabel → {"Drug concentration (as multiples of K_I)", "Relative growth rate"},
 PlotLabel → "Growth rate response to drug dose", FrameTicks → {logframeticks, Automatic}]
```



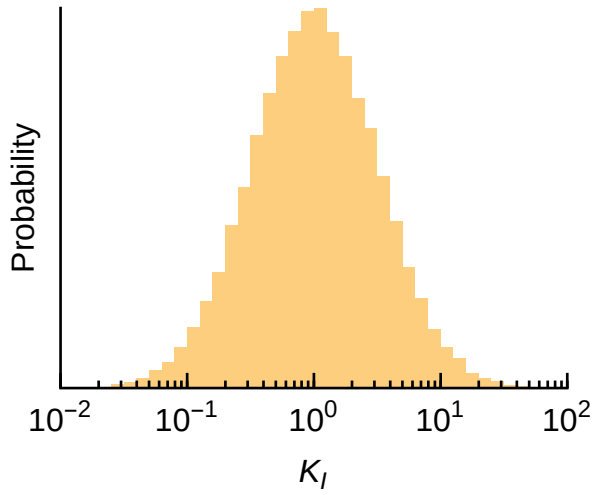
Modeling variation in drug sensitivity

Patient-to-patient variability in drug sensitivity is introduced in this model through variation in K_I. Note that although K_I takes the form of a binding affinity, this is not to suggest that variation in target binding affinity is the cause of patient-to-patient variability; rather, K_I serves as a convenient device to introduce the phenomenon of patient variability in drug response. We take this variability to be an initial assumption (based on observable facts) and explore its therapeutic consequences.

Based on analysis of human clinical trials (see Supplementary Figure S1), we take K_I to be log-normally distributed with standard deviation of approximately 0.5 on log-scale (that is, a half-decade).

Recalling that particular units are not relevant, for simplicity we take K_I to be distributed around a median of 1 (or 10^0)

```
StandardDeviationInDrugSensitivity = 0.5;
MedianLogDrugSensitivity = 0;

Histogram[
 Table[RandomVariate[NormalDistribution[MedianLogDrugSensitivity,
    StandardDeviationInDrugSensitivity]], {100000}], {-2, 2, 0.1}, ChartStyle → EdgeForm[None],
 Frame → {{True, False}, {True, False}}, Axes → False,
 FrameTicks → {{None, None}, {logframeticks, None}}, PlotRangePadding → None,
 FrameStyle → Directive[Black, Thickness[Medium]],
 BaseStyle → {FontFamily → "Arial", FontSize → 12}, FrameLabel → {"K_I", "Probability"},
 AspectRatio → 3 / 4, ImageSize → {{250}, {250}}, ImagePadding → {{50, 10}, {60, 10}},
 PlotRange → {{-2, 2}, {0, All}}]
```

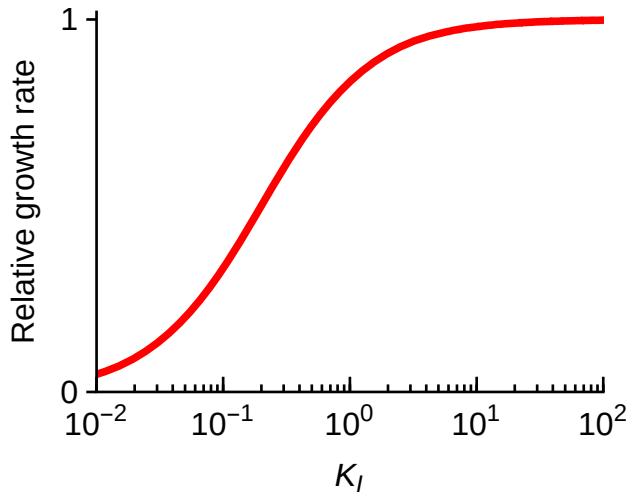


Drug concentration in a simulation can be chosen to be so high that all patients respond strongly, or so low that no patients respond. Here we choose a dose for which approximately half of tumors shrink, and half of tumors continue to grow, representing a clinically typical scenario wherein a fraction of patients' tumors respond to treatment.

```
DrugDose = 0.2;
```

Illustrating the effect of different KI values on drug response. Low KI indicates high drug sensitivity (strong growth inhibition), and high KI indicates low drug sensitivity (little to no inhibitory effect).

```
Plot[LogDrugInhibitionModel /. {LogDrug → Log[10, DrugDose], NH → 1}, {LogKI, -2, 2},
 Frame → {{True, False}, {True, False}}, Axes → {True, False},
 AxesStyle → Directive[Black, Thickness[Medium]],
 FrameTicks → {{Table[{i, i, {0, 0.02}}, {i, -1, 1, 1}], None}, {logframeticks, None}},
 PlotRangePadding → None, PlotRange → {{-2, 2}, {0, 1.02}},
 FrameStyle → Directive[Black, Thickness[Medium]],
 BaseStyle → {FontFamily → "Arial", FontSize → 12}, FrameLabel → {"K_I", "Relative growth rate"},
 AspectRatio → 3 / 4, ImageSize → {{250}, {250}}, ImagePadding → {{50, 10}, {60, 10}},
 PlotStyle → Directive[Red, Thickness[0.015]]]
```

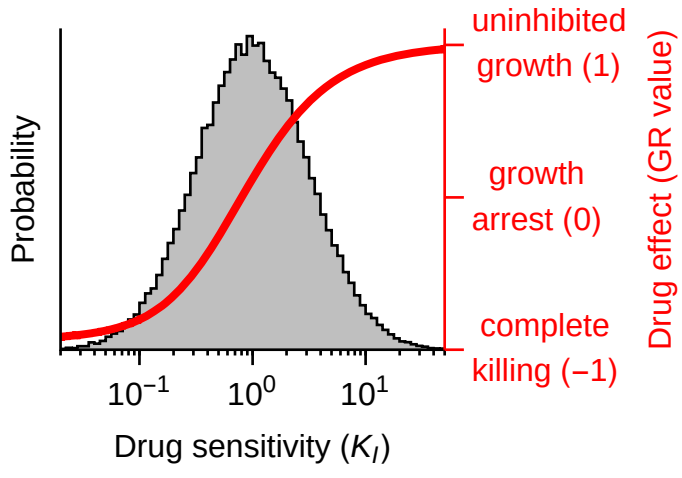


In the context of high rates of innate cell loss, a partial inhibition of tumor cell growth is sufficient for production to be balanced by loss, resulting in net tumor growth arrest. Here we plot the overall therapeutic response using the 'GR' (Growth Rate) metric, which converts relative growth rate to a normalized scale.
'RelativeGrowthRate' is the normalized change in cell number per cell division time; thus RelativeGrowthRate = 1 represents uninhibited growth, 0 represents growth arrest, and -1 describes a halving of the population per cell division time.

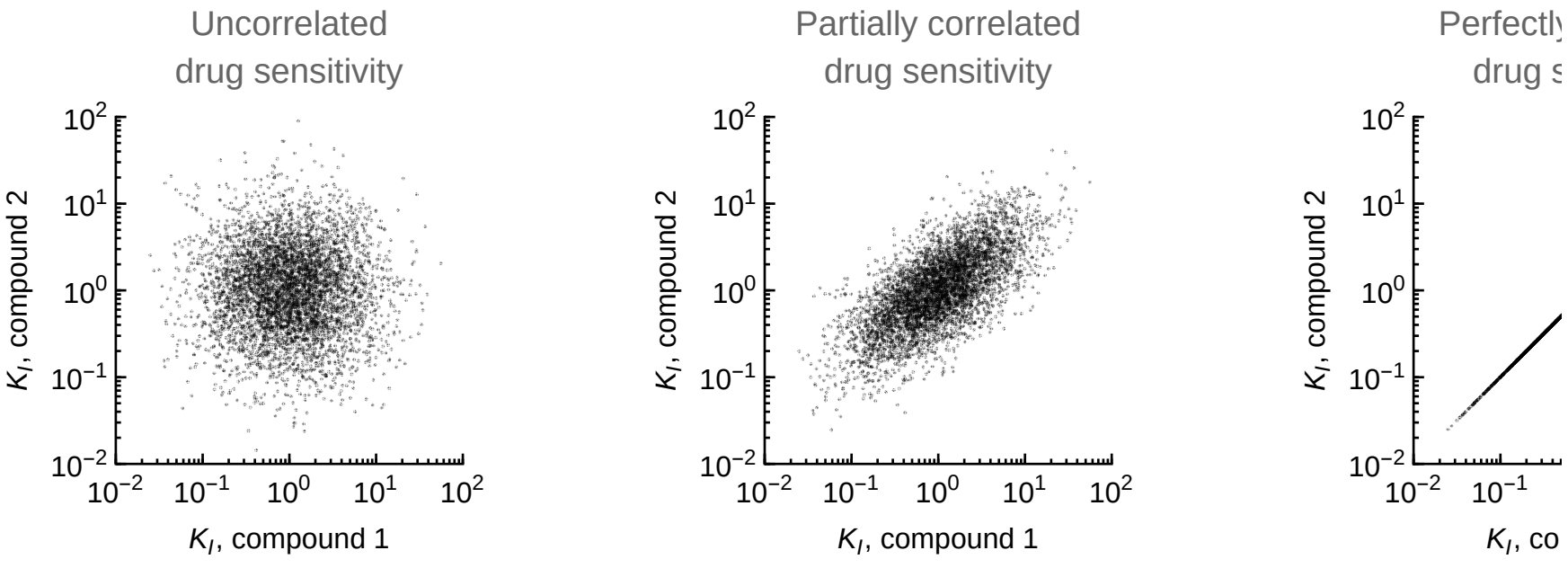
GR is then a metric that ranges from full, unhibited growth to complete tumor cell killing, on a scale that is normalized from 1 to -1, (1 = full growth, 0 = growth arrest, -1 = death of all cells). This scale is produced by the equation
$GR = 2^{\text{RelativeGrowthRate}} - 1$

Reference:
Hafner *et al*. Growth rate inhibition metrics correct for confounders in measuring sensitivity to cancer drugs. Nature Methods. 2016, **v13**:521-527

```
logframeticksDownward = Join[Table[{x, "10"^ToString[x], {0, 0.04}}, {x, -10, 10}],
   Flatten[Table[Table[{Log[10, x], "", {0, 0.015}}, {x, 1 * 10^y, 9 * 10^y, 1 * 10^y}], {y, -10, 10, 1}],
    1]];

LogNormallyDistributedKIvalues =
  Table[RandomVariate[NormalDistribution[MedianLogDrugSensitivity,
     StandardDeviationInDrugSensitivity]], {100000}];

CustomHistogramPlot[data_, bins_] :=
 Module[
  {countBorder = Partition[Riffle[Riffle[#1, #1[[2 ;;]]], Riffle[#2, #2]], 2] & @@
     HistogramList[data, bins, "Count"]},
  ListLinePlot[countBorder, Filling → Axis, FillingStyle → Directive[GrayLevel[0.75], Opacity[1]],
   PlotStyle -> Directive[Opacity[1], Black, AbsoluteThickness[1]],
   Frame → {{True, True}, {True, False}}, Axes → {False, False},
   FrameTicks →
    {{None, (Table[{i * 2000 + 2000, Style[ToString[i], Red], {0, 0.05}}, {i, -1, 1, 1}] /.
        {"0" → "growth\narrest (0)", "1" → Style["uninhibited\ngrowth (1)", LineSpacing → {4, 0}],
         "-1" → "complete\nkilling (-1)"})}, {logframeticksDownward, None}},
   PlotRangePadding → None,
   FrameStyle → {{Directive[GrayLevel[0.0], Thickness[Medium]],
      Directive[Red, Thickness[Medium]]},
     {Directive[Black, Thickness[Medium]], Directive[Black, Thickness[Medium]]}},
   BaseStyle → {FontFamily → "Arial", FontSize → 12},
   FrameLabel → {{Style["Probability", GrayLevel[0.]], Style["Drug effect (GR value)", Red]},
     {"Drug sensitivity (K_I)",}}, AspectRatio → 5 / 6, ImageSize → {{1000}, {200}},
   ImagePadding → {{50, 110}, {60, 20}}, PlotRange → {{Log[10, 0.02], Log[10, 50]}, {0, 4200}}]]

SensitivityDistributionAndDrugResponsePlot = Show[
  CustomHistogramPlot[LogNormallyDistributedKIvalues, {-1.7, 1.7, 0.05}]
  ,
  Plot[
   (2^(((LogDrugInhibitionModel /. {LogDrug → Log[10, DrugDose], NH → 1}) - CellLossRate) /
         (1 - CellLossRate)) - 1) * 2000 + 2000, {LogKI, Log[10, 0.02], Log[10, 50]},
   Frame → {{True, False}, {True, False}}, Axes → {True, False},
   AxesStyle → Directive[Black, Thickness[Medium]],
   FrameTicks → {{None, Table[{i, Style[ToString[i], Black], {0, 0.02}}, {i, -1, 1, 1}]},
     {logframeticks, None}}, PlotRangePadding → None, PlotRange → {{-2, 2}, {0, All}},
   FrameStyle → Directive[Black, Thickness[Medium]],
   BaseStyle → {FontFamily → "Arial", FontSize → 12},
   FrameLabel → {{"Drug sensitivity (K_I)",}, {"Probability", "GR"}}, AspectRatio → 3 / 4,
   ImageSize → {{250}, {250}}, ImagePadding → {{50, 10}, {60, 10}},
   PlotStyle → Directive[Red, AbsoluteThickness[3]]]
 ]

Export[NotebookDirectory[] <> "Figure 6B - KI distribution and therapeutic response.pdf",
  SensitivityDistributionAndDrugResponsePlot, "PDF"];
```


uninhibited
growth (1)
growth
arrest (0)
complete
killing (−1)
Drug effect (GR value)
Probability
10^−1
10^0
10^1
Drug sensitivity (K_I)

```
(* Generating joint KI distributions for drug 1 and drug 2,
with variable level of correlation. We subsequently explore the importance of
 correlation in drug sensitivity. *)
KIdistributionForDrug1 =
  Table[RandomVariate[NormalDistribution[MedianLogDrugSensitivity,
     StandardDeviationInDrugSensitivity]], {5000}];
KIdistributionForDrug2 =
  Table[RandomVariate[NormalDistribution[MedianLogDrugSensitivity,
     StandardDeviationInDrugSensitivity]], {5000}];
(* generating a correlated list with correlation specified by input variable ρ *)
CorrelatedKIs[ρ_] :=
  {KIdistributionForDrug1, (ρ * KIdistributionForDrug1 + (1 - ρ^2) KIdistributionForDrug2)}^T;

(* Illustrating uncorrelated drug sensitivity *)
UncorrelatedSensitivityPlot = ListPlot[CorrelatedKIs[0.], Frame → {{True, False}, {True, False}},
   FrameStyle → Directive[Black, Thickness[Medium]], Axes → False, AspectRatio → 1,
   PlotRange → {{-2, 2}, {-2, 2}}, PlotRangePadding → None,
   FrameStyle → Directive[Black, Thickness[Medium]],
   BaseStyle → {FontFamily → "Arial", FontSize → 12},
   FrameLabel → {"K_I, compound 1", "K_I, compound 2"}, AspectRatio → 3 / 4,
   ImageSize → {{500}, {250}}, ImagePadding → {{60, 10}, {60, 10}},
   FrameTicks → {{logframeticks, None}, {logframeticks, None}},
   PlotStyle → Directive[Black, Opacity[0.3], AbsolutePointSize[1.5]],
   PlotLabel → "Uncorrelated\ndrug sensitivity"];

(* Illustrating partially correlated drug sensitivity *)
PartiallyCorrelatedSensitivityPlot =
  ListPlot[CorrelatedKIs[0.6], Frame → {{True, False}, {True, False}},
   FrameStyle → Directive[Black, Thickness[Medium]], Axes → False, AspectRatio → 1,
   PlotRange → {{-2, 2}, {-2, 2}}, PlotRangePadding → None,
   FrameStyle → Directive[Black, Thickness[Medium]],
   BaseStyle → {FontFamily → "Arial", FontSize → 12},
   FrameLabel → {"K_I, compound 1", "K_I, compound 2"}, AspectRatio → 3 / 4,
   ImageSize → {{500}, {250}}, ImagePadding → {{60, 10}, {60, 10}},
   FrameTicks → {{logframeticks, None}, {logframeticks, None}},
   PlotStyle → Directive[Black, Opacity[0.3], AbsolutePointSize[1.5]],
   PlotLabel → "Partially correlated\ndrug sensitivity"];

(* Illustrating perfectly correlated drug sensitivity *)
PerfectlyCorrelatedSensitivityPlot =
  ListPlot[CorrelatedKIs[1.0], Frame → {{True, False}, {True, False}},
   FrameStyle → Directive[Black, Thickness[Medium]], Axes → False, AspectRatio → 1,
   PlotRange → {{-2, 2}, {-2, 2}}, PlotRangePadding → None,
   FrameStyle → Directive[Black, Thickness[Medium]],
   BaseStyle → {FontFamily → "Arial", FontSize → 12},
   FrameLabel → {"K_I, compound 1", "K_I, compound 2"}, AspectRatio → 3 / 4,
   ImageSize → {{500}, {250}}, ImagePadding → {{60, 10}, {60, 10}},
   FrameTicks → {{logframeticks, None}, {logframeticks, None}},
   PlotStyle → Directive[Black, Opacity[0.3], AbsolutePointSize[1.5]],
   PlotLabel → "Perfectly correlated\ndrug sensitivity"];

GraphicsRow[{UncorrelatedSensitivityPlot, PartiallyCorrelatedSensitivityPlot,
  PerfectlyCorrelatedSensitivityPlot}]
```



Modeling tumor growth and drug inhibition

This model describes the growth and drug responses of four populations of tumor cells:
(S) the majority of tumor cells with 'baseline' drug sensitivity.
(R1) a sub-population with greater resistance to the first of two drugs
(R2) a sub-population with greater resistance to the second of two drugs
(RR) a sub-population with greater resistance to both drugs

We first take resistance and cross-resistance frequencies from lineage-tracing experiments in a lung adenocarcinoma cell line, which measured pre-existing erlotinib resistance at a frequency of 500 cells per million, and pre-existing double-drug resistance (erlotinib and crizotinib) at 5 cells per million.

We are interested in describing the duration of progression free survival, in other words, time taken for tumor volume (cell count) to double.
Because the model is 'continuous' rather than discrete and stochastic, the initial tumor cell count has no effect in this model on the time taken to double. We take initial tumor cell count to be 10^{10}; in clinical reality different tumors will be different sizes at the start of of treatment, but in this model changing this parameter is inconsequential.

We use a numerical differential equation solver (NDSolve), applied to the following equation:

$$\frac{dp}{dt}(t) = g.p(t).\frac{1}{1+[drug]/K_I} - g.\phi.p(t),$$

g = growth rate
p(t) = tumor population at time 't'
[drug] = drug concentration
ϕ = cell loss factor
KI is the smaller (most sensitive) of K_I1 and K_I2, being the inhibitory constants of drug 1 and drug 2. This is the assumption of 'independent drug action' - each tumor cell's response is determined only by the one drug that is most effective against that cell.

Drug resistant sub-populations have their K_I values for the appropriate drug(s) increased by a factor of 10 (parameter 'rl' = resistance level)

Supplementary Figure S7A shows that different values of K_I, the magnitude of resistance (parameter 'rl'), and frequencies of resistance alter the shape of progression-free-survival curves

but do not alter the key results that independent drug action provides a significant benefit in a drug combination.

```
(* this function takes as its inputs
 KI for drug 1 (KI1),
KI for drug 2 (KI2),
ProgressionThreshold, being how large an increase in tumor volume is our output (2), and,
growthratenumber, which indicates which value from the list of randomly generated
 growth rates is being taken in this particular instance of the simulation. This
 takes integer values indicating a position in the previously generated list '
 DistributionOfGrowthRates'.
*)

ProgressionTime[KI1_, KI2_, growthrate_, ProgressionThreshold_, Correlation_] := Module[{},
  KI1internal = KI1;
  KI2internal = KI2;
  (*cell division rate *)
  gr = growthrate;
  (*spontaneous cell death rate*)
  celllossrate = CellLossRate; (* previously defined as 80% *)
  (* death rate = growth rate × cell loss factor *)
  dr = gr * celllossrate;
  (* rl = 'resistance level', being the fold-change in KI in drug resistant sub-populations *)
  rl = 10;

  (* pre-existing resistance frequencies *)
  (* resistance to drug 1 *) rf1 = 500 * 10^-6 * (1 - Correlation);
  (* resistance to drug 2 *) rf2 = 500 * 10^-6 * (1 - Correlation);
  (* resistance to both drugs *) rfCross = Max[{5 * 10^-6, 500 * 10^-6 * Correlation}];

  (* initial population size *)
  p0 = 10^10;
  (*drug dose, previously defined *)
  d = DrugDose;

  (* duration of numerical simulation *)
  SimulationDuration = 6; (*years*)

  (* simulating an untreated tumor *)
  mysimresultUntreated =
   NDSolve[{p'[t] == gr * p[t] - dr * p[t], p[0] == p0}, p, {t, 0, SimulationDuration * 365}][[1]];

  (* simulating growth of tumor cells with baseline drug sensitivity *)
  mysimresultS =
   NDSolve[
     {p'[t] ==
       gr * p[t] * DrugTreatmentOverTime[t, LogDrugInhibitionModel /.
            {LogDrug → Log[10, d], LogKI → Min[{Log[10, KI1internal], Log[10, KI2internal]}],
             NH → 1}] - dr * p[t], p[0] == p0}, p, {t, 0, SimulationDuration * 365}][[1]];
  (* simulating growth of subpopulation with resistance to drug 1 *)
  mysimresultR1 =
   NDSolve[
     {p'[t] ==
       gr * p[t] * DrugTreatmentOverTime[t, LogDrugInhibitionModel /.
            {LogDrug → Log[10, d], LogKI → Min[{Log[10, KI1internal * rl], Log[10, KI2internal]}],
             NH → 1}] - dr * p[t], p[0] == p0 * rf1}, p, {t, 0, SimulationDuration * 365}][[1]];
  (* simulating growth of subpopulation with resistance to drug 2 *)
  mysimresultR2 =
```

```
 NDSolve[
   {p'[t] ==
     gr * p[t] * DrugTreatmentOverTime[t, LogDrugInhibitionModel /.
          {LogDrug → Log[10, d], LogKI → Min[{Log[10, KI1internal], Log[10, KI2internal * rl]}],
           NH → 1}] - dr * p[t], p[0] == p0 * rf2}, p, {t, 0, SimulationDuration * 365}][[1]];
(* simulating growth of subpopulation with resistance to both drugs *)
mysimresultRR =
 NDSolve[
   {p'[t] ==
     gr * p[t] * DrugTreatmentOverTime[t, LogDrugInhibitionModel /.
          {LogDrug → Log[10, d],
           LogKI → Min[{Log[10, KI1internal * rl], Log[10, KI2internal * rl]}], NH → 1}] - dr * p[t],
    p[0] == p0 * rfCross}, p, {t, 0, SimulationDuration * 365}][[1]];

(* Summing up the total population size at each time; sum of populations S, R1,
R2, RR. Table has an entry at every 2 days. *)
PopulationOverTimeTable =
 Table[{t, (p[t] /. mysimresultS) + (p[t] /. mysimresultR1) + (p[t] /. mysimresultR2) +
    (p[t] /. mysimresultRR)}, {t, 0, (SimulationDuration - 0.1) * 365, 2}];

  (* If progression threshold is not reached within the duration of the simulation,
  return a number larger than the duration of simulation
   (this can be used to identify such events) *)
  If[PopulationOverTimeTable[[-1, 2]] < ProgressionThreshold,
   Return[(SimulationDuration + 1) * 365]];

  (* final output: the earliest time at which the total population size surpasses
     the 'progression threshold', taken in this file to be twice the initial population,
  though it could be varied *)
  Select[PopulationOverTimeTable, #[[2]] > ProgressionThreshold &][[1, 1]]
 ]
```

‘SimulatePopulation’ executes the above simulation (ProgressionTime) for each individual in a population of 200.
It takes a single input parameter, being the correlation between KI for drug 1 and KI for drug 2

```
SimulatePopulation[Correlation_] := Module[{},

  KI1table =
   Table[RandomVariate[NormalDistribution[MedianLogDrugSensitivity,
      StandardDeviationInDrugSensitivity]], {1000}];
KI2table =
   Table[RandomVariate[NormalDistribution[MedianLogDrugSensitivity,
      StandardDeviationInDrugSensitivity]], {1000}];

KIJointDistribution = {KI1table, (Correlation * KI1table + (1 - Correlation^2) KI2table)}^T;

logKI1 = 10^KIJointDistribution[[All, 1]];
logKI2 = 10^KIJointDistribution[[All, 2]];

NumberOfTumorsSimulated = 200;
ProgressionThreshold = 2 * 10^10;

(* simulating progression free survival time for no treatment. No treatment is
 implemented by setting KI1 and KI2 to equal 1000;
at this magnitude neither drug has any inhibitory effect on growth *)
UntreatedProgressionTimes[Correlation] =
```

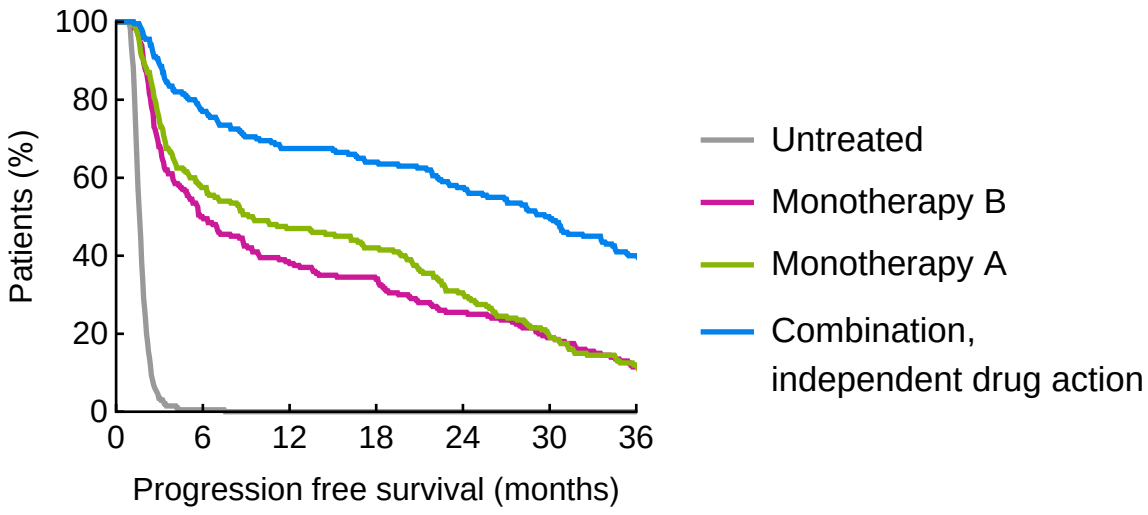
```
 Parallelize[Table[ProgressionTime[1000, 1000, DistributionOfGrowthRates[[i]],
    ProgressionThreshold, Correlation], {i, 1, NumberOfTumorsSimulated}]];
(* simulating progression free survival time for treatment by monotherapy #1. The
 absence of monotherapy #2 is implemented by setting KI2 to equal 1000;
at this magnitude drug 2 has no inhibitory effect on growth *)
Monotherapy1ProgressionTimes[Correlation] =
 Parallelize[Table[ProgressionTime[logKI1[[i]], 1000, DistributionOfGrowthRates[[i]],
    ProgressionThreshold, Correlation], {i, 1, NumberOfTumorsSimulated}]];
(* simulating progression free survival time for treatment by monotherapy #2. The
 absence of monotherapy #1 is implemented by setting KI1 to equal 1000;
at this magnitude drug 2 has no inhibitory effect on growth *)
Monotherapy2ProgressionTimes[Correlation] =
 Parallelize[Table[ProgressionTime[1000, logKI2[[i]], DistributionOfGrowthRates[[i]],
    ProgressionThreshold, Correlation], {i, 1, NumberOfTumorsSimulated}]];
(* simulating progression free survival time for treatment by a combination of
 monotherapy #1 and #2 *)
CombinationProgressionTimes[Correlation] =
 Parallelize[Table[ProgressionTime[logKI1[[i]], logKI2[[i]], DistributionOfGrowthRates[[i]],
    ProgressionThreshold, Correlation], {i, 1, NumberOfTumorsSimulated}]];
```

```
(* Plotting survival functions *)
SurvivalPlot = ListPlot[{
   Table[{x, SurvivalFunction[EmpiricalDistribution[UntreatedProgressionTimes[Correlation]]][
      x]}, {x, 0, 5 * 365, 2}],
   Table[
    {x, SurvivalFunction[EmpiricalDistribution[Monotherapy2ProgressionTimes[Correlation]]][
      x]}, {x, 0, 5 * 365, 2}],
   Table[
    {x, SurvivalFunction[EmpiricalDistribution[Monotherapy1ProgressionTimes[Correlation]]][
      x]}, {x, 0, 5 * 365, 2}],
   Table[
    {x, SurvivalFunction[EmpiricalDistribution[CombinationProgressionTimes[Correlation]]][
      x]}, {x, 0, 5 * 365, 2}]
  },
  Joined → True, FrameTicks → {Table[{i, i * 2 / 61, {0.015, 0}}, {i, 0, 2000, 3 / 2 * 122}],
    Table[{i, 100 * i, {0.015, 0}}, {i, 0, 1, 1 / 5}]}, PlotRange → {{0, 36 * 30.5}, {0, 1}},
  Frame → {{True, False}, {True, False}}, FrameStyle → Directive[Black, Thickness[Medium]],
  BaseStyle → {FontFamily → "Arial", FontSize → 12}, AspectRatio → 3 / 4,
  FrameLabel → {"Progression free survival (months)", "Patients (%)"},
  ImageSize → {{250}, {250}}, ImagePadding → {{45, 10}, {45, 10}},
```

```
   PlotStyle → {Directive[GrayLevel[0.6], AbsoluteThickness[2]],
     Directive[RGBColor[0.8, 0.1, 0.6], AbsoluteThickness[2]],
     Directive[ColorData[3, 4], AbsoluteThickness[2]],
     Directive[ColorData[3, 6], AbsoluteThickness[2]]},
   PlotLegends → {"Untreated", "Monotherapy B", "Monotherapy A",
     "Combination,\nindependent drug action"}]
 ]
```

Executing

```
(* this command allows legends to be included in exported images without interfering
 with the proper size of the plot. *)
SetOptions[$FrontEndSession, PrintingStyleEnvironment → "Working"]

SimulatePopulation[0]
```




```
Export[NotebookDirectory[] <>
   "Progression free survival, combination with independent drug action.pdf", %, "PDF"];
```

Spider plots, for monotherapy

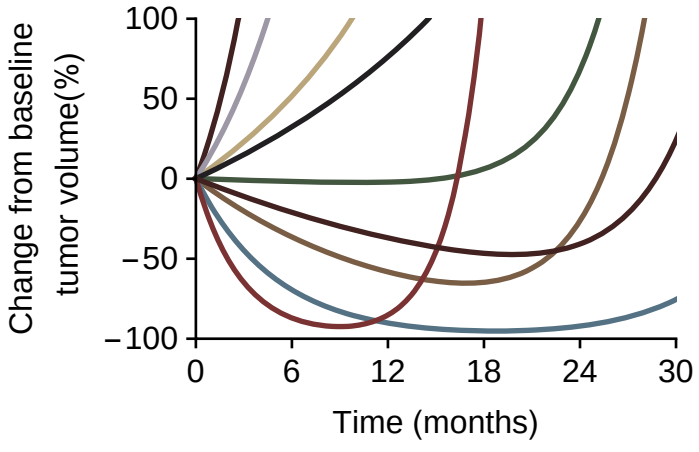
Because growth rates and drug sensitivities (KI) are randomly drawn from distributions, reproducible example trajectories can only be generated here by ‘hard-coding’ some sample values.

A small set of growth rates was generated by
Table[Max[{1/100,RandomVariate[NormalDistribution[6.9/100,2.1/100]]}],{50}];

A small set of KI values was generated by
10^Table[RandomVariate[NormalDistribution[MedianLogDrugSensitivity,StandardDeviationInDrugSensitivity]],{50}]

These parameters generate 50 different tumor kinetic trajectories. A few of these trajectories have been selected here, non-randomly, to illustrate the variety of different kinetic trajectories.

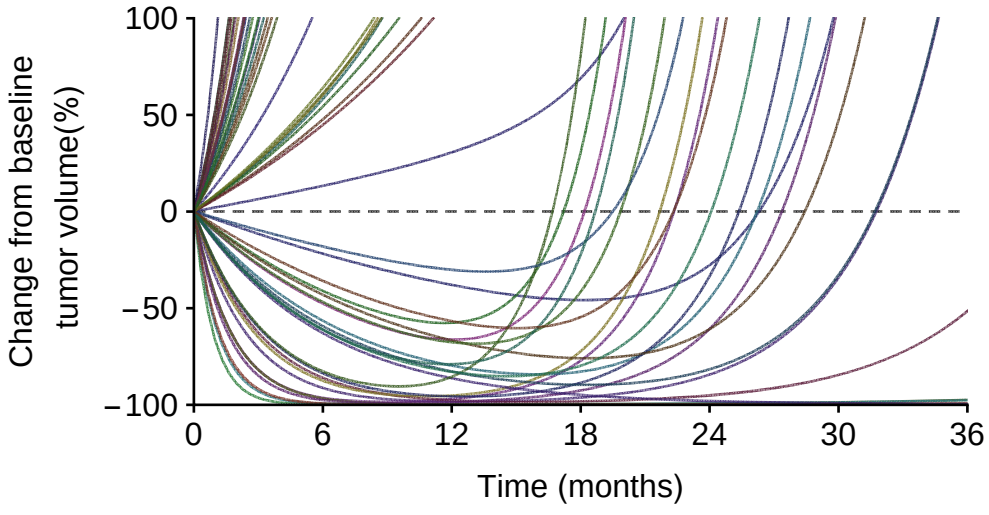
```
ASmallSetOfGrowthRates = {0.052`, 0.06`, 0.057`, 0.086`, 0.068`, 0.095`, 0.057`, 0.053`,
   0.045`, 0.033`, 0.083`, 0.084`, 0.061`, 0.094`, 0.092`, 0.077`, 0.088`, 0.1`, 0.098`,
   0.055`, 0.059`, 0.098`, 0.048`, 0.043`, 0.028`, 0.108`, 0.1`, 0.029`, 0.062`, 0.058`,
   0.045`, 0.063`, 0.088`, 0.079`, 0.038`, 0.057`, 0.121`, 0.075`, 0.054`, 0.101`, 0.063`,
   0.069`, 0.069`, 0.046`, 0.047`, 0.113`, 0.058`, 0.094`, 0.09`, 0.067`};

ASmallSetOfKIvalues = {5.41`, 3.84`, 0.62`, 0.95`, 1.83`, 3.46`, 0.79`, 1.06`, 0.38`,
   4.06`, 4.1`, 3.54`, 0.61`, 2.85`, 3.2`, 3.82`, 1.45`, 1.3`, 1.25`, 0.96`, 3.63`, 0.43`,
   0.68`, 2.53`, 3.96`, 1.14`, 1.69`, 0.32`, 1.38`, 0.4`, 0.96`, 1.68`, 0.63`, 2.1`,
   0.34`, 1.79`, 0.15`, 15.78`, 0.2`, 0.12`, 0.42`, 1.90`, 26.7`, 0.24`, 0.89`, 1.25`,
   4.62`, 0.93`, 0.8`, 2.62`};

Do[
 ProgressionTime[ASmallSetOfKIvalues[[SimulatedTumorNumber]], 1000,
  ASmallSetOfGrowthRates[[SimulatedTumorNumber]], ProgressionThreshold, 0];
 TotalPopulationOverTime[SimulatedTumorNumber] =
  Table[
   {t,
    ((p[t] /. mysimresultS) + (p[t] /. mysimresultR1) + (p[t] /. mysimresultR2) +
        (p[t] /. mysimresultRR)) / 10^10 - 1}, {t, 0, (SimulationDuration - 0.5) * 365, 10}];
 SPopulationOverTime[SimulatedTumorNumber] =
  Table[{t, (p[t] /. mysimresultS) / 10^10 - 1}, {t, 0, (SimulationDuration - 0.5) * 365, 10}];
 R1PopulationOverTime[SimulatedTumorNumber] =
  Table[{t, (p[t] /. mysimresultR1) / 10^10 - 1}, {t, 0, (SimulationDuration - 0.5) * 365, 10}];
 R2PopulationOverTime[SimulatedTumorNumber] =
  Table[{t, (p[t] /. mysimresultR2) / 10^10 - 1}, {t, 0, (SimulationDuration - 0.5) * 365, 10}];
 RRPopulationOverTime[SimulatedTumorNumber] =
  Table[{t, (p[t] /. mysimresultRR) / 10^10 - 1}, {t, 0, (SimulationDuration - 0.5) * 365, 10}];

 , {SimulatedTumorNumber, 1, 50}]

(* a subset was selected (non-randomly) to illustrate the diversity of different
 treatment outcomes *)
ListPlot[Table[TotalPopulationOverTime[stn], {stn, {1, 3, 4, 7, 9, 10, 20, 22, 23}}],
 PlotRange → {{0, 30 * 30.5}, {-1, 1}}, Joined → True,
 PlotStyle →
  Table[Directive[Blend[{ColorData[16, i], ColorConvert[ColorData[16, i], "Grayscale"]}, 0.7],
    Opacity[1], AbsoluteThickness[2]], {i, {1, 2, 3, 4, 6, 7, 8, 9, 10}}],
 Frame → {{True, False}, {True, False}}, Axes → {False, False}, Ticks → None,
 PlotRangePadding → {{0, 0}, {0.0, 0}}, FrameStyle → Directive[Black, Thickness[Medium]],
 AxesStyle -> Directive[Black, Thickness[Medium]],
 BaseStyle → {FontFamily → "Arial", FontSize → 12},
 FrameTicks → {{Table[{i, 100 * i, {0, 0.02}}, {i, -1, 1, 1 / 2}], None},
   {Table[{i, i * 2 / 61, {0, 0.02}}, {i, 0, 2000, 6 / 4 * 122}], None}}, AspectRatio → 2 / 3,
 ImageSize → {{1000}, {200}}, ImagePadding → {{100, 10}, {60, 20}},
 FrameLabel → {"Time (months)", "Change from baseline\n tumor volume(%)"},
 Prolog → {Dashing[{0.015, 0.01}], Opacity[0.7], GrayLevel[0.], Thickness[Medium](*,
   Line[{{0,0},{2*366,0}}]*)}]

Export[NotebookDirectory[] <> "Figure 6D, Monotherapy spider plot.pdf", %, "PDF"];
```



A larger batch produced by randomly drawn parameters

```
Do[
 ProgressionTime[logKI1[[SimulatedTumorNumber]], 1000,
  DistributionOfGrowthRates[[SimulatedTumorNumber]], ProgressionThreshold, 0];
 TotalPopulationOverTime[SimulatedTumorNumber] =
  Table[
   {t,
    ((p[t] /. mysimresultS) + (p[t] /. mysimresultR1) + (p[t] /. mysimresultR2) +
        (p[t] /. mysimresultRR)) / 10^10 - 1}, {t, 0, (SimulationDuration - 0.5) * 365, 10}];
SPopulationOverTime[SimulatedTumorNumber] =
 Table[{t, (p[t] /. mysimresultS) / 10^10 - 1}, {t, 0, (SimulationDuration - 0.5) * 365, 10}];
R1PopulationOverTime[SimulatedTumorNumber] =
 Table[{t, (p[t] /. mysimresultR1) / 10^10 - 1}, {t, 0, (SimulationDuration - 0.5) * 365, 10}];
R2PopulationOverTime[SimulatedTumorNumber] =
 Table[{t, (p[t] /. mysimresultR2) / 10^10 - 1}, {t, 0, (SimulationDuration - 0.5) * 365, 10}];
RRPopulationOverTime[SimulatedTumorNumber] =
 Table[{t, (p[t] /. mysimresultRR) / 10^10 - 1}, {t, 0, (SimulationDuration - 0.5) * 365, 10}];

, {SimulatedTumorNumber, 1, 200}]
```

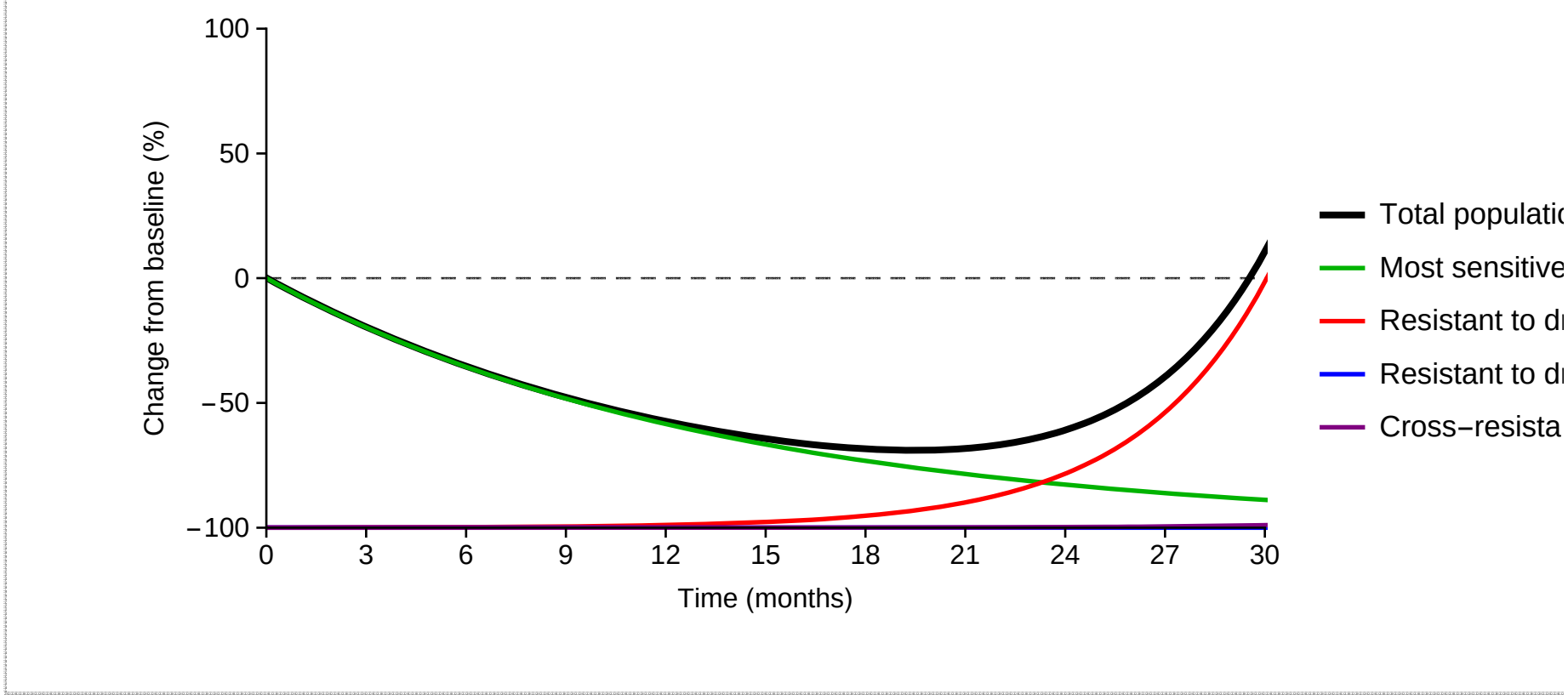
```
ListPlot[Table[TotalPopulationOverTime[SimulatedTumorNumber], {SimulatedTumorNumber, 50, 100}],
 PlotRange → {{0, 3 * 366}, {-1, 1}}, Joined → True,
 PlotStyle → Table[Directive[Hue[RandomReal[{0, 1}], 0.7, RandomReal[{0.3, 0.5}]],
    Opacity[0.7], Thickness[Medium]], {200}], Frame → {{True, False}, {True, False}},
 Axes → {False, False}, Ticks → None, PlotRangePadding → None,
 FrameStyle → Directive[Black, Thickness[Medium]],
 AxesStyle -> Directive[Black, Thickness[Medium]],
 BaseStyle → {FontFamily → "Arial", FontSize → 12},
 FrameTicks → {{Table[{i, 100 * i, {0, 0.01}}, {i, -1, 1, 1 / 2}], None},
   {Table[{i, i * 2 / 61, {0, 0.01}}, {i, 0, 2000, 3 / 2 * 122}], None}}, AspectRatio → 1 / 2,
 ImageSize → {{400}, {500}}, ImagePadding → {{100, 10}, {60, 20}},
 FrameLabel → {"Time (months)", "Change from baseline\n tumor volume(%)"},
 Prolog → {Dashing[{0.015, 0.01}], Opacity[0.7], GrayLevel[0.], Thickness[Medium],
   Line[{{0, 0}, {3 * 366, 0}}]}]
```



This interface allows the user to scroll through different individual tumors and inspect the growth (or inhibition) of its sub - populations over time.

```
Manipulate[
 ListPlot[{TotalPopulationOverTime[sample], SPopulationOverTime[sample],
   R1PopulationOverTime[sample], R2PopulationOverTime[sample], RRPopulationOverTime[sample]},
  PlotRange → {{0, 30 * 30.5}, {-1, 1}}, Joined → True,
  PlotStyle → Join[{Directive[Black, Opacity[1], AbsoluteThickness[3]]},
    Table[Directive[col, Opacity[1], AbsoluteThickness[2]],
     {col, {Darker[Green, 0.3], Red, Blue, Purple}}]], Frame → {{True, False}, {True, False}},
  Axes → {False, False}, Ticks → None, PlotRangePadding → {{0, 0}, {0.0, 0}},
  FrameStyle → Directive[Black, Thickness[Medium]],
  AxesStyle -> Directive[Black, Thickness[Medium]],
  BaseStyle → {FontFamily → "Arial", FontSize → 12},
  FrameTicks → {{Table[{i, 100 * i, {0, 0.01}}, {i, -1, 1, 1 / 2}], None},
    {Table[{i, i * 2 / 61, {0, 0.01}}, {i, 0, 2000, 3 / 4 * 122}], None}}, AspectRatio → 1 / 2,
  ImageSize → {{1000}, {300}}, ImagePadding → {{100, 10}, {60, 20}},
  FrameLabel → {"Time (months)", "Change from baseline (%)"},
  Prolog → {Dashing[{0.015, 0.01}], Opacity[0.7], GrayLevel[0.], Thickness[Medium],
    Line[{{0, 0}, {3 * 366, 0}}]},
  PlotLegends → {"Total population", "Most sensitive (S)", "Resistant to drug 1 (R1)",
    "Resistant to drug 2 (R2)", "Cross-resistant (RR)"}]
, {sample, 1, 200, 1}]
```

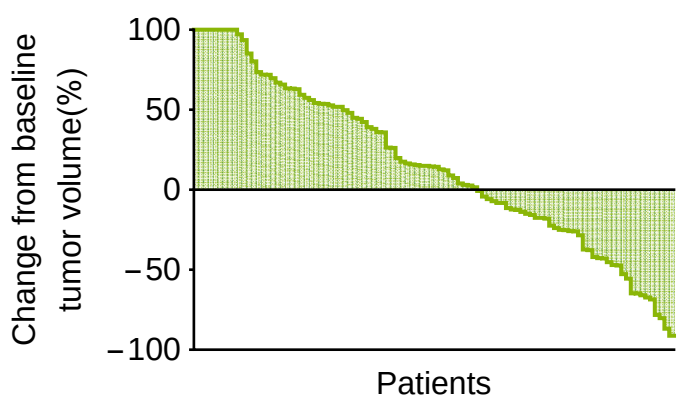
sample



Waterfall plots, for monotherapy

```
VolumeChangeAfterMonotherapy[SimulatedTumorNumber_] := Module[{},

  (* which time, in weeks, will we evaluate the change in tumor volume? *)
  FollowupTimeInWeeks = 8;

  (* executing a simulation for a single tumor *)
  ProgressionTime[logKI1[[SimulatedTumorNumber]], 1000,
   DistributionOfGrowthRates[[SimulatedTumorNumber]], ProgressionThreshold, 0];

  (* selecting the first entry in the 'PopulationOverTimeTable' that is after the
   follow up time *)
  PopulationAtFollowUp = Select[PopulationOverTimeTable, #[[1]] <= FollowupTimeInWeeks * 7 &][[
    -1, 2]];
  InitialPopulation = 10^10;
  (* calculating the relative change in size *)
  BestFractionalChangeWithinFollowUpTime = PopulationAtFollowUp / InitialPopulation
  ]

WaterFallDistribution = Table[VolumeChangeAfterMonotherapy[stn], {stn, 1, 200}];

NumberOfPatients = 100;
(* creating a line to track the waterfall plot *)
StartBarPositions =
  {Range[NumberOfPatients] - 0.5,
    Map[Min[{#, 1.0}] &, Reverse[Sort[WaterFallDistribution[[1 ;; 0 + NumberOfPatients]] - 1]]]}^T;
EndBarPositions =
  {Range[NumberOfPatients] + 0.5,
    Map[Min[{#, 1.0}] &, Reverse[Sort[WaterFallDistribution[[1 ;; 0 + NumberOfPatients]] - 1]]]}^T;
```

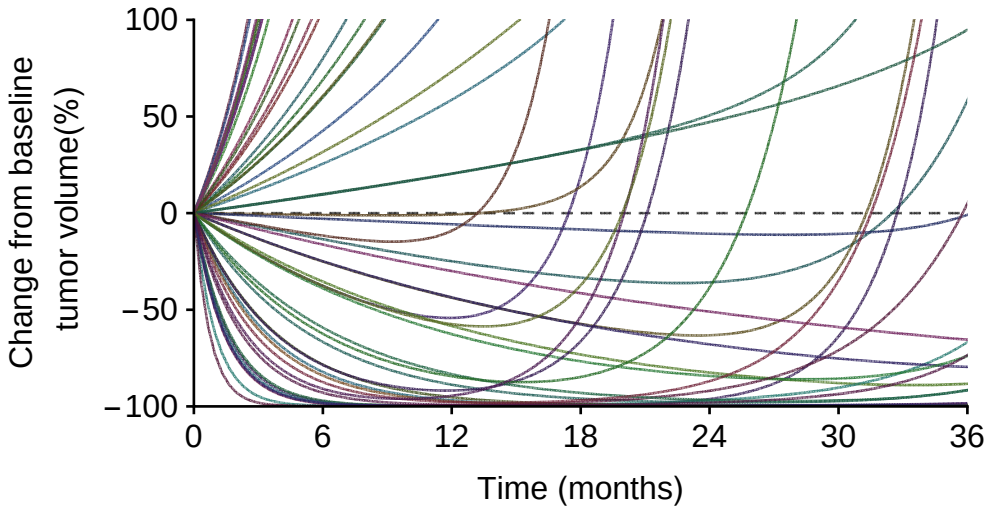
```
Show[
 BarChart[
  Map[Min[{#, 1.0}] &, Reverse[Sort[WaterFallDistribution[[1 ;; 0 + NumberOfPatients]] - 1]]],
  PlotRange → {{1 / 2, NumberOfPatients + 1 / 2}, {-1, 1}}, Frame → {{True, False}, {True, False}},
  Axes → {False, False}, Ticks → None, PlotRangePadding → None, ChartLabels → None,
  FrameStyle → Directive[Black, Thickness[Medium]],
  AxesStyle -> Directive[Black, Thickness[Medium]],
  BaseStyle → {FontFamily → "Arial", FontSize → 12},
  FrameTicks → {{Table[{i, 100 * i, {0, 0.01}}, {i, -1, 1, 1 / 2}], None}, {None, None}},
  ChartStyle →
   Directive[EdgeForm[Directive[ColorData[31, 8], AbsoluteThickness[0.5], Opacity[0.15]]],
    Opacity[0.3], ColorData[3, 4]], BarSpacing → None, AspectRatio → 2 / 3,
  ImageSize → {{1000}, {200}}, ImagePadding → {{100, 10}, {60, 20}},
  FrameLabel → {"Patients", "Change from baseline\ntumor volume(%)"},
  Epilog → {Opacity[1], Black, Thickness[Medium], Line[{{1 / 2, 0}, {1 / 2 + NumberOfPatients, 0}}]
    (*,Gray,Dashing[{0.02,0.015}],Line[{{1/2,-1/3},{1/2+NumberOfPatients,-1/3}}],
    Line[{{1/2,1/3},{1/2+NumberOfPatients,1/3}}]*)}]
 ,
 ListPlot[Riffle[StartBarPositions, EndBarPositions], Joined → True,
  PlotStyle → Directive[AbsoluteThickness[1.5], ColorData[3, 4]]]
]

Export[NotebookDirectory[] <> "Figure 6C, Monotherapy waterfall plot.pdf", %, "PDF"];
```



Spider plots, for combination therapy

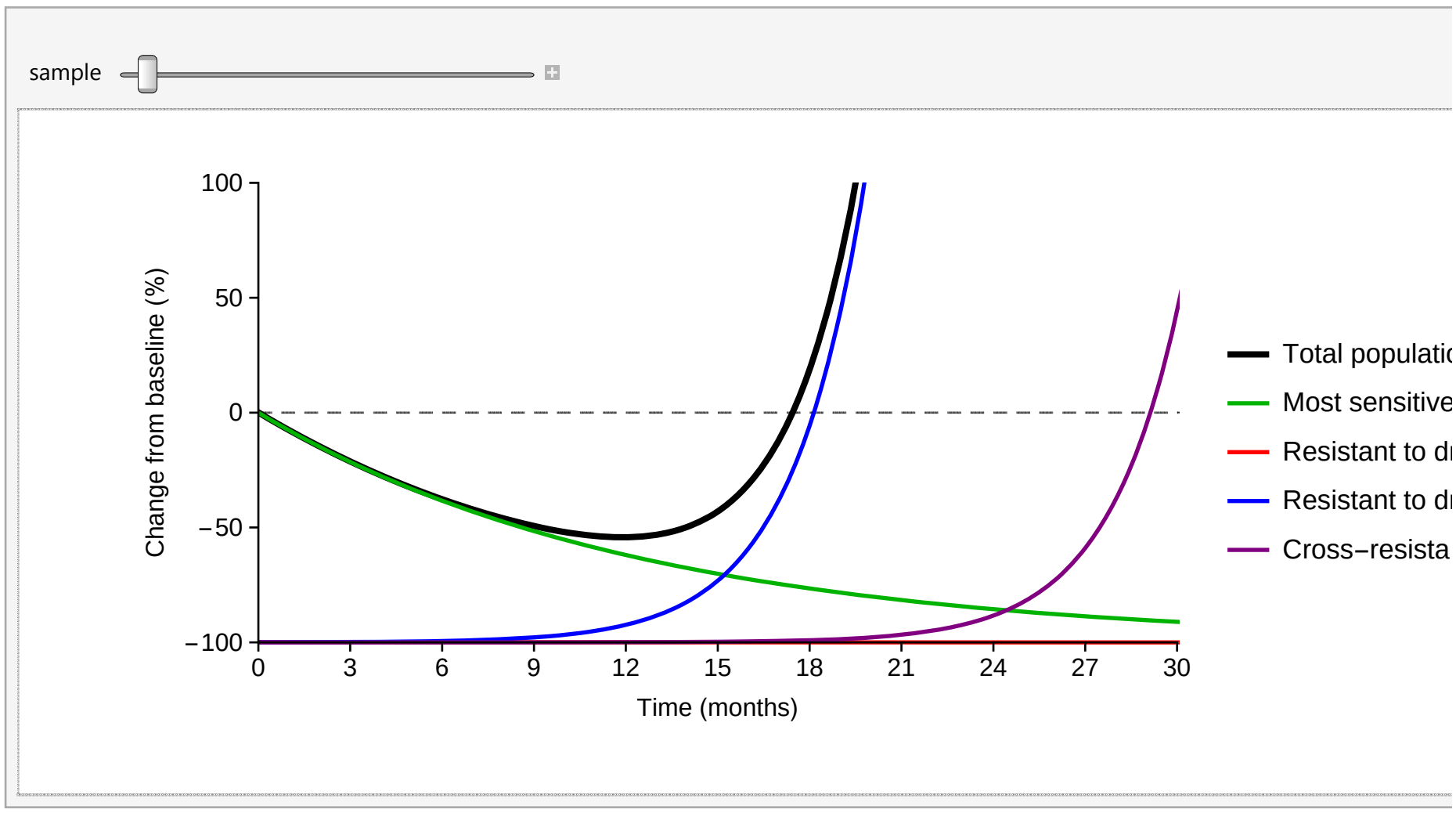
A large batch produced by randomly drawn parameters

```
Do[
 ProgressionTime[logKI1[[SimulatedTumorNumber]], logKI2[[SimulatedTumorNumber]],
  DistributionOfGrowthRates[[SimulatedTumorNumber]], ProgressionThreshold, 0];
 TotalPopulationOverTimeCombination[SimulatedTumorNumber] =
  Table[
   {t,
    ((p[t] /. mysimresultS) + (p[t] /. mysimresultR1) + (p[t] /. mysimresultR2) +
        (p[t] /. mysimresultRR)) / 10^10 - 1}, {t, 0, (SimulationDuration - 0.5) * 365, 10}];
 SPopulationOverTimeCombination[SimulatedTumorNumber] =
  Table[{t, (p[t] /. mysimresultS) / 10^10 - 1}, {t, 0, (SimulationDuration - 0.5) * 365, 10}];
 R1PopulationOverTimeCombination[SimulatedTumorNumber] =
  Table[{t, (p[t] /. mysimresultR1) / 10^10 - 1}, {t, 0, (SimulationDuration - 0.5) * 365, 10}];
 R2PopulationOverTimeCombination[SimulatedTumorNumber] =
  Table[{t, (p[t] /. mysimresultR2) / 10^10 - 1}, {t, 0, (SimulationDuration - 0.5) * 365, 10}];
 RRPopulationOverTimeCombination[SimulatedTumorNumber] =
  Table[{t, (p[t] /. mysimresultRR) / 10^10 - 1}, {t, 0, (SimulationDuration - 0.5) * 365, 10}];

 , {SimulatedTumorNumber, 1, 200}]

ListPlot[Table[TotalPopulationOverTimeCombination[stn], {stn, 1, 50}],
 PlotRange → {{0, 3 * 366}, {-1, 1}}, Joined → True,
 PlotStyle → Table[Directive[Hue[RandomReal[{0, 1}], 0.7, RandomReal[{0.3, 0.5}]],
    Opacity[0.7], Thickness[Medium]], {200}], Frame → {{True, False}, {True, False}},
 Axes → {False, False}, Ticks → None, PlotRangePadding → None,
 FrameStyle → Directive[Black, Thickness[Medium]],
 AxesStyle -> Directive[Black, Thickness[Medium]],
 BaseStyle → {FontFamily → "Arial", FontSize → 12},
 FrameTicks → {{Table[{i, 100 * i, {0, 0.01}}, {i, -1, 1, 1 / 2}], None},
   {Table[{i, i * 2 / 61, {0, 0.01}}, {i, 0, 2000, 3 / 2 * 122}], None}}, AspectRatio → 1 / 2,
 ImageSize → {{400}, {500}}, ImagePadding → {{100, 10}, {60, 20}},
 FrameLabel → {"Time (months)", "Change from baseline\n tumor volume(%)"},
 Prolog → {Dashing[{0.015, 0.01}], Opacity[0.7], GrayLevel[0.], Thickness[Medium],
   Line[{{0, 0}, {3 * 366, 0}}]}]
```



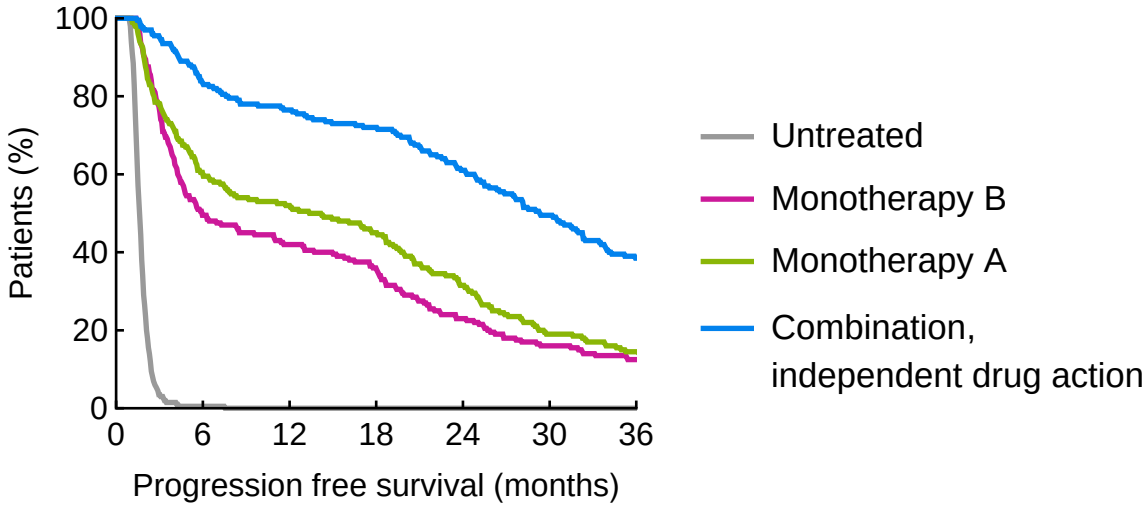
This interface allows the user to scroll through different individual tumors and inspect the growth (or inhibition) of its sub - populations over time.

```
Manipulate[
 ListPlot[{TotalPopulationOverTimeCombination[sample], SPopulationOverTimeCombination[sample],
   R1PopulationOverTimeCombination[sample], R2PopulationOverTimeCombination[sample],
   RRPopulationOverTimeCombination[sample]}, PlotRange → {{0, 30 * 30.5}, {-1, 1}},
  Joined → True,
  PlotStyle → Join[{Directive[Black, Opacity[1], AbsoluteThickness[3]]},
    Table[Directive[col, Opacity[1], AbsoluteThickness[2]],
     {col, {Darker[Green, 0.3], Red, Blue, Purple}}]], Frame → {{True, False}, {True, False}},
  Axes → {False, False}, Ticks → None, PlotRangePadding → {{0, 0}, {0.0, 0}},
  FrameStyle → Directive[Black, Thickness[Medium]],
  AxesStyle -> Directive[Black, Thickness[Medium]],
  BaseStyle → {FontFamily → "Arial", FontSize → 12},
  FrameTicks → {{Table[{i, 100 * i, {0, 0.01}}, {i, -1, 1, 1 / 2}], None},
    {Table[{i, i * 2 / 61, {0, 0.01}}, {i, 0, 2000, 3 / 4 * 122}], None}}, AspectRatio → 1 / 2,
  ImageSize → {{1000}, {300}}, ImagePadding → {{100, 10}, {60, 20}},
  FrameLabel → {"Time (months)", "Change from baseline (%)"},
  Prolog → {Dashing[{0.015, 0.01}], Opacity[0.7], GrayLevel[0.], Thickness[Medium],
    Line[{{0, 0}, {3 * 366, 0}}]},
  PlotLegends → {"Total population", "Most sensitive (S)", "Resistant to drug 1 (R1)",
    "Resistant to drug 2 (R2)", "Cross-resistant (RR)"}]
, {sample, 1, 200, 1}]
```

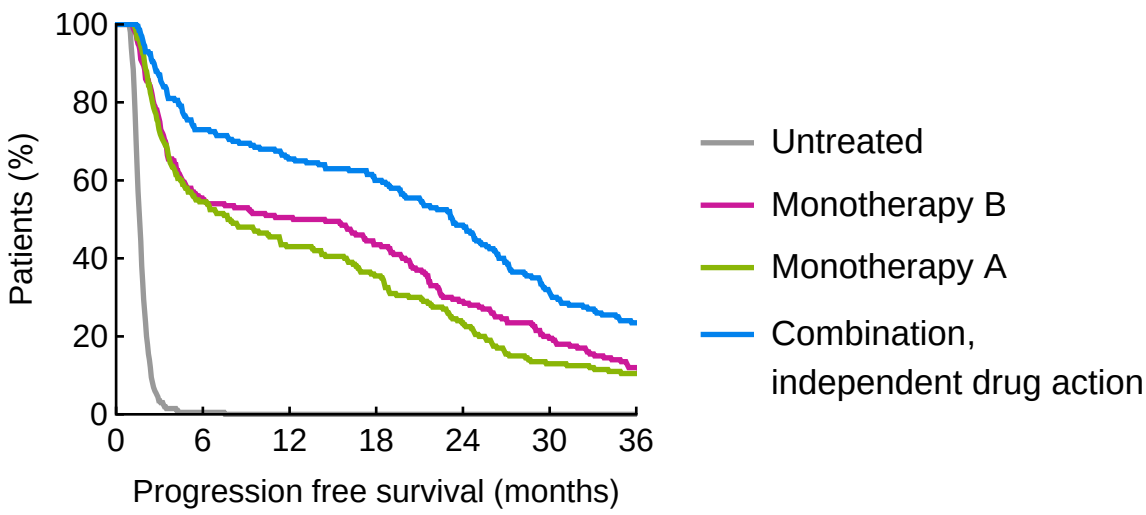


Modeling consequence of different levels of correlation in drug response

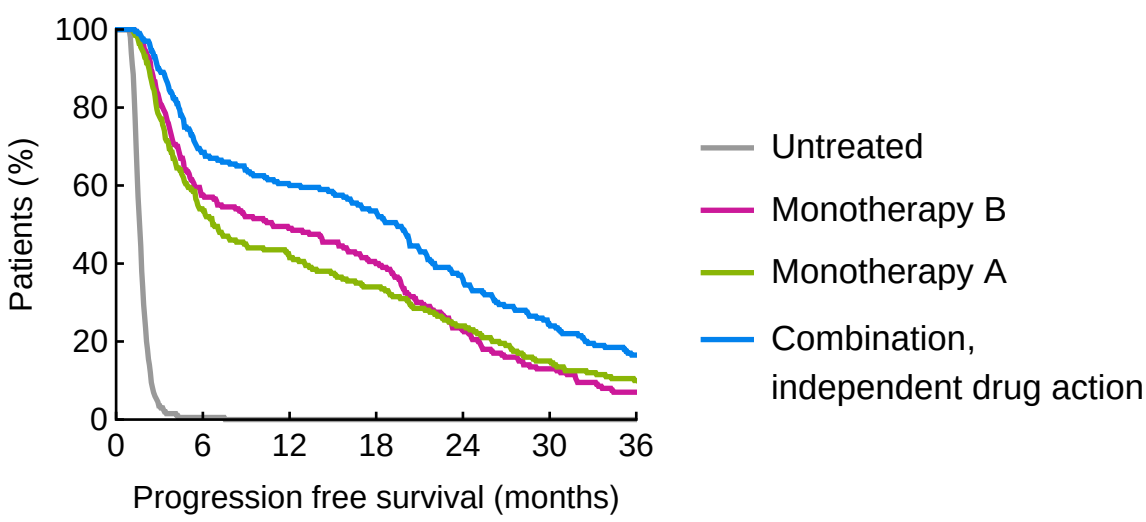
```
(* simulation of independent action with response correlation = 0.0 *)
SimulatePopulation[0]
```



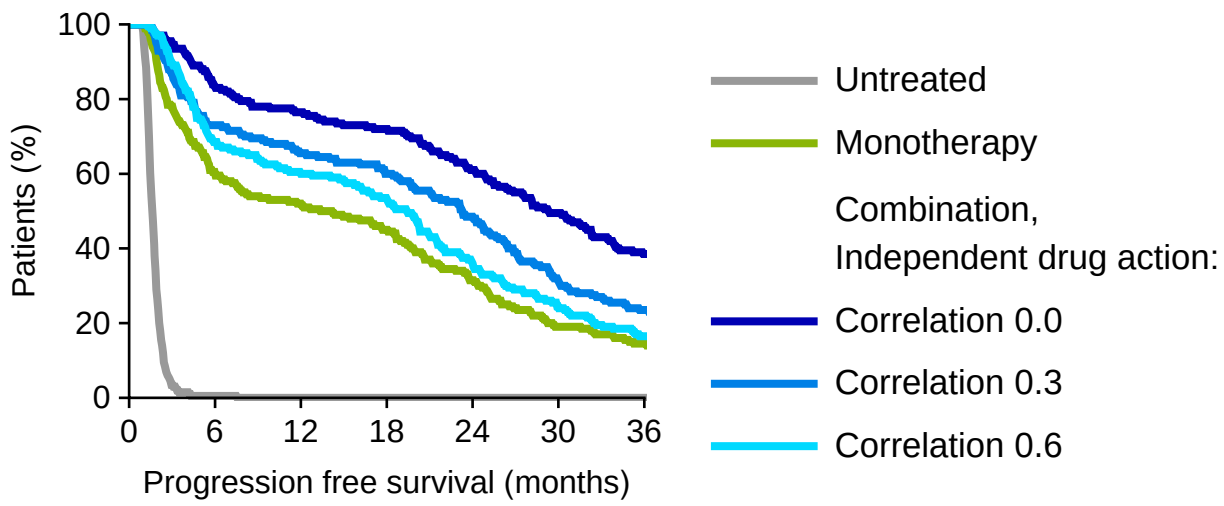

```
(* simulation of independent action with response correlation = 0.3 *)
SimulatePopulation[0.3]
```




```
(* simulation of independent action with response correlation = 0.6 *)
SimulatePopulation[0.6]
```



```
IndependenceGradientPlot = ListPlot[{
   Table[{x, SurvivalFunction[EmpiricalDistribution[UntreatedProgressionTimes[0]]][x]},
    {x, 0, 4 * 365, 2}],
   (*Table[{x,SurvivalFunction[EmpiricalDistribution[Monotherapy2ProgressionTimes[0]]][x]},
    {x,0,4*365,2}],*)
   Table[{x, SurvivalFunction[EmpiricalDistribution[Monotherapy1ProgressionTimes[0]]][x]},
    {x, 0, 4 * 365, 2}],
   ,
   Table[{x, SurvivalFunction[EmpiricalDistribution[CombinationProgressionTimes[0]]][x]},
    {x, 0, 4 * 365, 2}],
   Table[{x, SurvivalFunction[EmpiricalDistribution[CombinationProgressionTimes[0.3]]][x]},
    {x, 0, 4 * 365, 2}],
   Table[{x, SurvivalFunction[EmpiricalDistribution[CombinationProgressionTimes[0.6]]][x]},
    {x, 0, 4 * 365, 2}]
  },
  Joined → True,
  FrameTicks → {{Table[{i, ToString[100 * i], {0, 0.02}}, {i, 0, 1, 1 / 5}], None},
    {Table[{i, i * 2 / 61, {0, 0.02}}, {i, 0, 2000, 3 / 2 * 122}], None}},
  PlotRange → {{0, 61 * 18}, {0, 1}}, Frame → {{True, False}, {True, False}},
  FrameStyle → Directive[Black, Thickness[Medium]],
  BaseStyle → {FontFamily → "Arial", FontSize → 12}, AspectRatio → 2.9 / 4,
  FrameLabel → {"Progression free survival (months)", Rotate["Patients (%)", 0]},
  ImageSize → {{1000}, {200}}, ImagePadding → {{60, 10}, {50, 10}},
  PlotStyle → {Directive[GrayLevel[0.6], AbsoluteThickness[3]],
    (*Directive[RGBColor[0.8,0.1,0.6],AbsoluteThickness[3]],*)
    Directive[ColorData[3, 4], AbsoluteThickness[3]], None,
    Directive[RGBColor[0, 0, 0.7](*RGBColor[0.67,0.1,0.67]*), Dashing[None],
     AbsoluteThickness[3]], Directive[RGBColor[0, 0.5, 0.9](*RGBColor[0.67,0.1,0.67]*),
     Dashing[None], AbsoluteThickness[3]],
    Directive[RGBColor[0, 0.85, 1](*RGBColor[0.67,0.1,0.67]*), Dashing[None],
     AbsoluteThickness[3]]},
  PlotLegends → Placed[{"Untreated", (*"Monotherapy B",*)"Monotherapy"(*<>" A"*),
     Style["Combination,\nIndependent drug action:", LineSpacing → {0, 0}],
     "Correlation 0.0", "Correlation 0.3", "Correlation 0.6"}, Right]]
```




```
Export[NotebookDirectory[] <> "Figure 6E, independent, PFS.pdf", %, "PDF"];
```

Modeling ‘additive’ or ‘synergistic’ drug combinations

Experiments in cell culture commonly quantify the degree of ‘synergy’ in a combination according to the models of Chou & Talalay or of Loewe which, by measuring the dose response to a fixed ratio combination or by isolobogram analysis (respectively), quantify the efficacy of a combination in comparison to higher or lower concentrations of monotherapy. A drug combination is additive if their combined effect equals the effect expected by summing their potency-normalized doses (for example, [A] / $IC50_A$, and [B] / $IC50_B$) on a common dose-response curve. A drug combination is

synergistic if their combined effect is greater than expected by the same measure.

Here we simulate a drug combination which behaves according to this understanding of drug additivity / synergism, by treated the combination as simply a higher dose of a monotherapy. This describes the scenario of perfect correlation in response (full cross-resistance) between the two drugs.

With two drugs acting as one, we only track two sub-populations: baseline level of drug sensitivity (S) and drug-resistant (R1).

References:

Chou & Talalay. Quantitative analysis of dose-effect relationships: the combined effects of multiple drugs or enzyme inhibitors. Adv. Enzyme Regul. 1984, **v22**:25-55

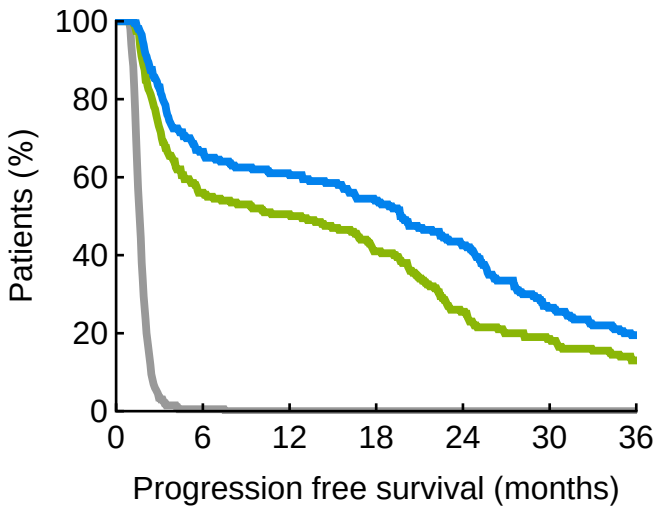
Loewe S. The problem of synergism and antagonism of combined drugs. Arzneimittelforschung. 1953, **v3**:285-90

```
ProgressionTimeSynergy[KI1_, DoseChange_, growthrate_, ProgressionThreshold_] := Module[{},
  KI1internal = KI1;
  (*cell division rate *)
  gr = growthrate;
  (*spontaneous cell death rate*)
  celllossrate = CellLossRate; (* previously defined as 80% *)
  (* death rate = growth rate × cell loss factor *)
  dr = gr * celllossrate;
  (* rl = 'resistance level', being the fold-change in KI in drug resistant sub-populations *)
  rl = 10;

  (* pre-existing resistance frequencies *)
  (* resistance to drug 1 *) rf1 = 500 * 10^-6;

  (* initial population size *)
  p0 = 10^10;
  (* Previously defined drug dose is multiplied by new parameter 'DoseChange' which
   describes how much more potent are two drugs in combination *)
  d = DrugDose * DoseChange;

  (* duration of numerical simulation *)
  SimulationDuration = 6; (*years*)

  (* simulating an untreated tumor *)
  mysimresultUntreated =
   NDSolve[{p'[t] == gr * p[t] - dr * p[t], p[0] == p0}, p, {t, 0, SimulationDuration * 365}][[1]];
  (* simulating growth of tumor cells with baseline drug sensitivity *)
  mysimresultS =
   NDSolve[
     {p'[t] ==
       gr * p[t] * DrugTreatmentOverTime[t, LogDrugInhibitionModel /.
            {LogDrug → Log[10, d], LogKI → Log[10, KI1internal], NH → 1}] - dr * p[t], p[0] == p0},
     p, {t, 0, SimulationDuration * 365}][[1]];
  (* simulating growth of tumor cells with drug resistance *)
  mysimresultR1 =
   NDSolve[
     {p'[t] ==
       gr * p[t] * DrugTreatmentOverTime[t, LogDrugInhibitionModel /.
            {LogDrug → Log[10, d], LogKI → Log[10, KI1internal * rl], NH → 1}] - dr * p[t],
      p[0] == p0 * rf1}, p, {t, 0, SimulationDuration * 365}][[1]];

  (* Summing up the total population size at each time; sum of populations S,
  R1. Table has an entry at every 2 days. *)
  PopulationOverTimeTable = Table[{t, (p[t] /. mysimresultS) + (p[t] /. mysimresultR1)},
    {t, 0, (SimulationDuration - 0.5) * 365, 2}];

  (* If progression threshold is not reached within the duration of the simulation,
  return a number larger than the duration of simulation
   (this can be used to identify such events) *)
  If[PopulationOverTimeTable[[-1, 2]] < ProgressionThreshold,
   Return[(SimulationDuration + 1) * 365]];

  (* final output: the earliest time at which the total population size surpasses
     the 'progression threshold', taken in this file to be twice the initial population,
  though it could be varied *)
  Select[PopulationOverTimeTable, #[[2]] > ProgressionThreshold &][[1, 1]]
]
```

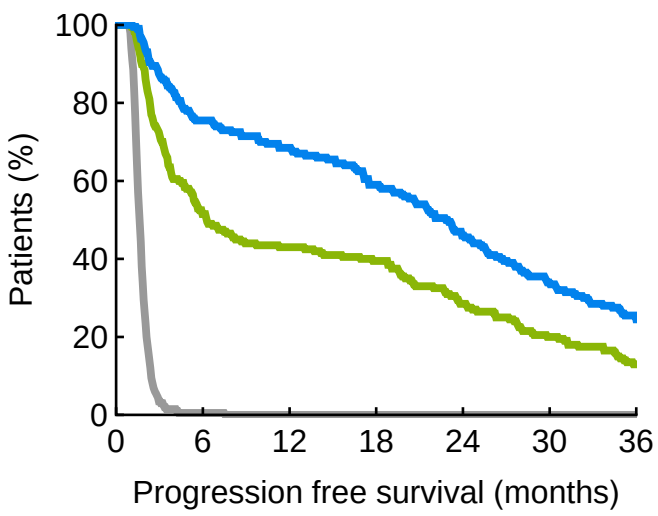
Executing the above simulation for a population of 200 patients

```
SimulatePopulationSynergy[DoseChange_] := Module[{},

  KI1Table = Table[RandomVariate[NormalDistribution[0, StandardDeviationInDrugSensitivity]],
    {1000}];

  logKI1 = 10^KI1Table;

  NumberOfTumorsSimulated = 200;
  ProgressionThreshold = 2 * 10^10;

  UntreatedProgressionTimesSynergy[DoseChange] =
   Parallelize[Table[ProgressionTimeSynergy[1000, 1, DistributionOfGrowthRates[[i]],
      ProgressionThreshold], {i, 1, NumberOfTumorsSimulated}]];
  MonotherapyProgressionTimesSynergy[DoseChange] =
   Parallelize[Table[ProgressionTimeSynergy[logKI1[[i]], 1, DistributionOfGrowthRates[[i]],
      ProgressionThreshold], {i, 1, NumberOfTumorsSimulated}]];
  CombinationProgressionTimesSynergy[DoseChange] =
   Parallelize[Table[ProgressionTimeSynergy[logKI1[[i]], DoseChange, DistributionOfGrowthRates[[i]],
     ProgressionThreshold], {i, 1, NumberOfTumorsSimulated}]];

 SurvivalPlotSynergy[DoseChange] = ListPlot[{
    Table[
     {x,
      SurvivalFunction[EmpiricalDistribution[UntreatedProgressionTimesSynergy[DoseChange]]][
       x]}, {x, 0, 5 * 365, 2}],
    Table[
     {x,
      SurvivalFunction[EmpiricalDistribution[MonotherapyProgressionTimesSynergy[DoseChange]]][
       x]}, {x, 0, 5 * 365, 2}],
    Table[
     {x,
      SurvivalFunction[EmpiricalDistribution[CombinationProgressionTimesSynergy[DoseChange]]][
       x]}, {x, 0, 5 * 365, 2}]
   },
   Joined → True, FrameTicks → {Table[{i, i * 2 / 61, {0.015, 0}}, {i, 0, 2000, 3 / 2 * 122}],
     Table[{i, 100 * i, {0.015, 0}}, {i, 0, 1, 1 / 5}]}, PlotRange → {{0, 36 * 30.5}, {0, 1}},
   Frame → {{True, False}, {True, False}}, FrameStyle → Directive[Black, Thickness[Medium]],
   BaseStyle → {FontFamily → "Arial", FontSize → 12}, AspectRatio → 3 / 4,
   FrameLabel → {"Progression free survival (months)", "Patients (%)"},
   ImageSize → {{250}, {250}}, ImagePadding → {{45, 10}, {45, 10}},
   PlotStyle → {Directive[GrayLevel[0.6], Thickness[0.015]],
     Directive[ColorData[3, 4], Thickness[0.015]],
     Directive[ColorData[3, 6], Thickness[0.015]]}]
]
```

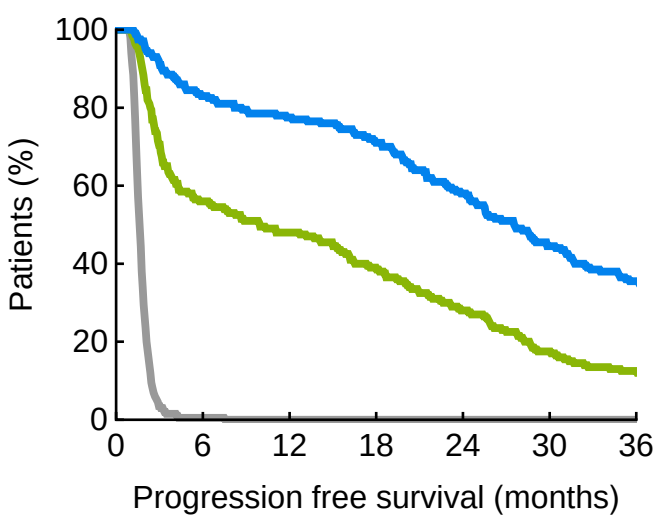
```
(* simulation of 'synergy' equivalent to 1.5× monotherapy dose *)
SimulatePopulationSynergy[1.5]
```



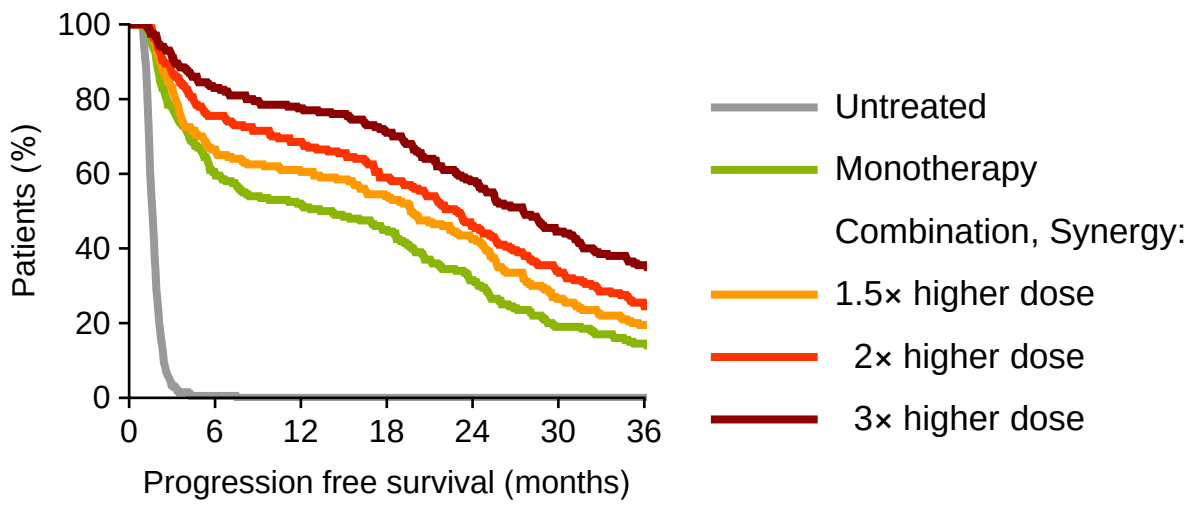

```
(* simulation of 'synergy' equivalent to 2× monotherapy dose *)
SimulatePopulationSynergy[2]
```




```
(* simulation of 'synergy' equivalent to 3× monotherapy dose *)
SimulatePopulationSynergy[3]
```



```
SynergyGradientPlot = ListPlot[{
   Table[{x, SurvivalFunction[EmpiricalDistribution[UntreatedProgressionTimes[0]]][x]},
    {x, 0, 4 * 365, 2}],
   (*Table[{x,SurvivalFunction[EmpiricalDistribution[Monotherapy2ProgressionTimes[0]]][x]},
    {x,0,4*365,2}],*)
   Table[{x, SurvivalFunction[EmpiricalDistribution[Monotherapy1ProgressionTimes[0]]][x]},
    {x, 0, 4 * 365, 2}],
   ,
   Table[{x, SurvivalFunction[EmpiricalDistribution[CombinationProgressionTimesSynergy[1.5]]][
      x]}, {x, 0, 4 * 365, 2}],
   Table[{x, SurvivalFunction[EmpiricalDistribution[CombinationProgressionTimesSynergy[2]]][
      x]}, {x, 0, 4 * 365, 2}],
   Table[{x, SurvivalFunction[EmpiricalDistribution[CombinationProgressionTimesSynergy[3]]][
      x]}, {x, 0, 4 * 365, 2}]
  },
  Joined → True,
  FrameTicks → {{Table[{i, ToString[100 * i], {0, 0.02}}, {i, 0, 1, 1 / 5}], None},
    {Table[{i, i * 2 / 61, {0, 0.02}}, {i, 0, 2000, 3 / 2 * 122}], None}},
  PlotRange → {{0, 61 * 18}, {0, 1}}, Frame → {{True, False}, {True, False}},
  FrameStyle → Directive[Black, Thickness[Medium]],
  BaseStyle → {FontFamily → "Arial", FontSize → 12}, AspectRatio → 2.9 / 4,
  FrameLabel → {"Progression free survival (months)", Rotate["Patients (%)", 0]},
  ImageSize → {{1000}, {200}}, ImagePadding → {{60, 10}, {50, 10}},
  PlotStyle → {Directive[GrayLevel[0.6], AbsoluteThickness[3]],
    (*Directive[RGBColor[0.8,0.1,0.6],AbsoluteThickness[3]],*)
    Directive[ColorData[3, 4], AbsoluteThickness[3]], None,
    Directive[RGBColor[1, 0.6, 0](*RGBColor[0.67,0.1,0.67]*), Dashing[None],
     AbsoluteThickness[3]], Directive[RGBColor[1, 0.2, 0](*RGBColor[0.67,0.1,0.67]*),
     Dashing[None], AbsoluteThickness[3]],
    Directive[RGBColor[0.55, 0, 0](*RGBColor[0.67,0.1,0.67]*), Dashing[None],
     AbsoluteThickness[3]]},
  PlotLegends → Placed[{"Untreated", (*"Monotherapy B",*)"Monotherapy"(*<>" A"*),
     "Combination, Synergy:", "1.5× higher dose", "  2× higher dose", "  3× higher dose"},
    Right]]
```



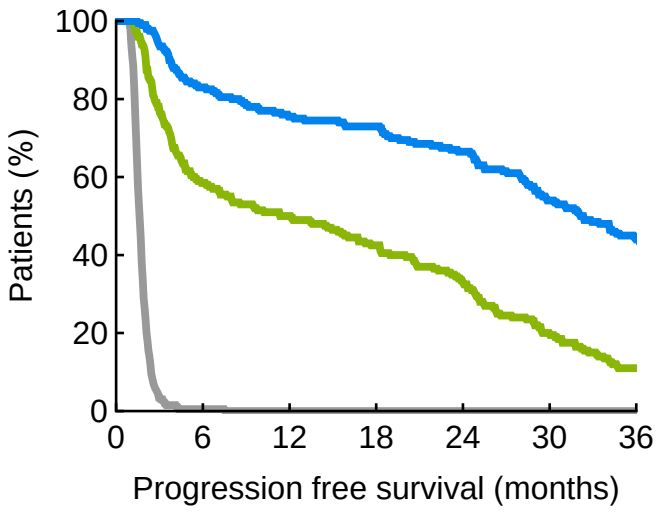

```
Export[NotebookDirectory[] <> "Figure 6E, synergy, PFS.pdf", %, "PDF"];
```

Independent drug action and additive-or-synergistic drug interactions are not mutually exclusive - they can act simultaneously

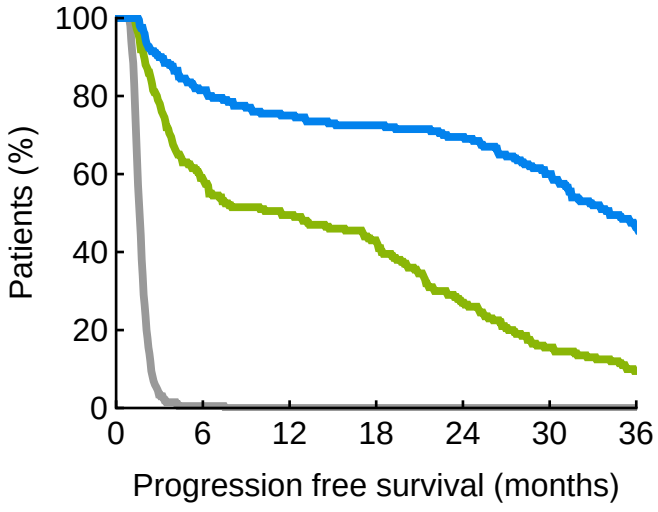
First, we examine the effect of a two drug combination that are partially correlated in response, and have a small increase in effective dose when co-administered (1.4×; in effect sub-additive)

```
DrugDose = 0.2;

ProgressionTimeMixedEffects[KI1_, KI2_, growthrate_, DoseChange_, ProgressionThreshold_] :=
 Module[{},
  KI1internal = KI1;
  KI2internal = KI2;

  gr = growthrate;

  celllossrate = CellLossRate; (* 80% cell loss rate *)
  dr = gr * celllossrate;
  rl = 10;
  rf1 = 500 * 10^-6;
  rf2 = 500 * 10^-6;
  rfCross = 5 * 10^-6;
  p0 = 10^10;
  d = DrugDose * DoseChange;
  SimulationDuration = 6; (*years*)
mysimresultUntreated =
 NDSolve[{p'[t] == gr * p[t] - dr * p[t], p[0] == p0}, p, {t, 0, SimulationDuration * 365}][[1]];
mysimresultS =
 NDSolve[
   {p'[t] ==
     gr * p[t] * DrugTreatmentOverTime[t, LogDrugInhibitionModel /.
          {LogDrug → Log[10, d], LogKI → Min[{Log[10, KI1internal], Log[10, KI2internal]}],
           NH → 1}] - dr * p[t], p[0] == p0}, p, {t, 0, SimulationDuration * 365}][[1]];
mysimresultR1 =
 NDSolve[
   {p'[t] ==
     gr * p[t] * DrugTreatmentOverTime[t, LogDrugInhibitionModel /.
          {LogDrug → Log[10, d], LogKI → Min[{Log[10, KI1internal * rl], Log[10, KI2internal]}],
           NH → 1}] - dr * p[t], p[0] == p0 * rf1}, p, {t, 0, SimulationDuration * 365}][[1]];
mysimresultR2 =
 NDSolve[
   {p'[t] ==
     gr * p[t] * DrugTreatmentOverTime[t, LogDrugInhibitionModel /.
          {LogDrug → Log[10, d], LogKI → Min[{Log[10, KI1internal], Log[10, KI2internal * rl]}],
           NH → 1}] - dr * p[t], p[0] == p0 * rf2}, p, {t, 0, SimulationDuration * 365}][[1]];
mysimresultRR =
 NDSolve[
   {p'[t] ==
     gr * p[t] * DrugTreatmentOverTime[t, LogDrugInhibitionModel /.
          {LogDrug → Log[10, d],
           LogKI → Min[{Log[10, KI1internal * rl], Log[10, KI2internal * rl]}], NH → 1}] - dr * p[t],
    p[0] == p0 * rfCross}, p, {t, 0, SimulationDuration * 365}][[1]];

  PopulationOverTimeTable =
   Table[{t, (p[t] /. mysimresultS) + (p[t] /. mysimresultR1) + (p[t] /. mysimresultR2) +
      (p[t] /. mysimresultRR)}, {t, 0, (SimulationDuration - 0.5) * 365, 2}];

  If[PopulationOverTimeTable[[-1, 2]] < ProgressionThreshold,
   Return[(SimulationDuration + 1) * 365]];

  Select[PopulationOverTimeTable, #[[2]] > ProgressionThreshold &][[1, 1]]
 ]

(* this simulation takes two
```

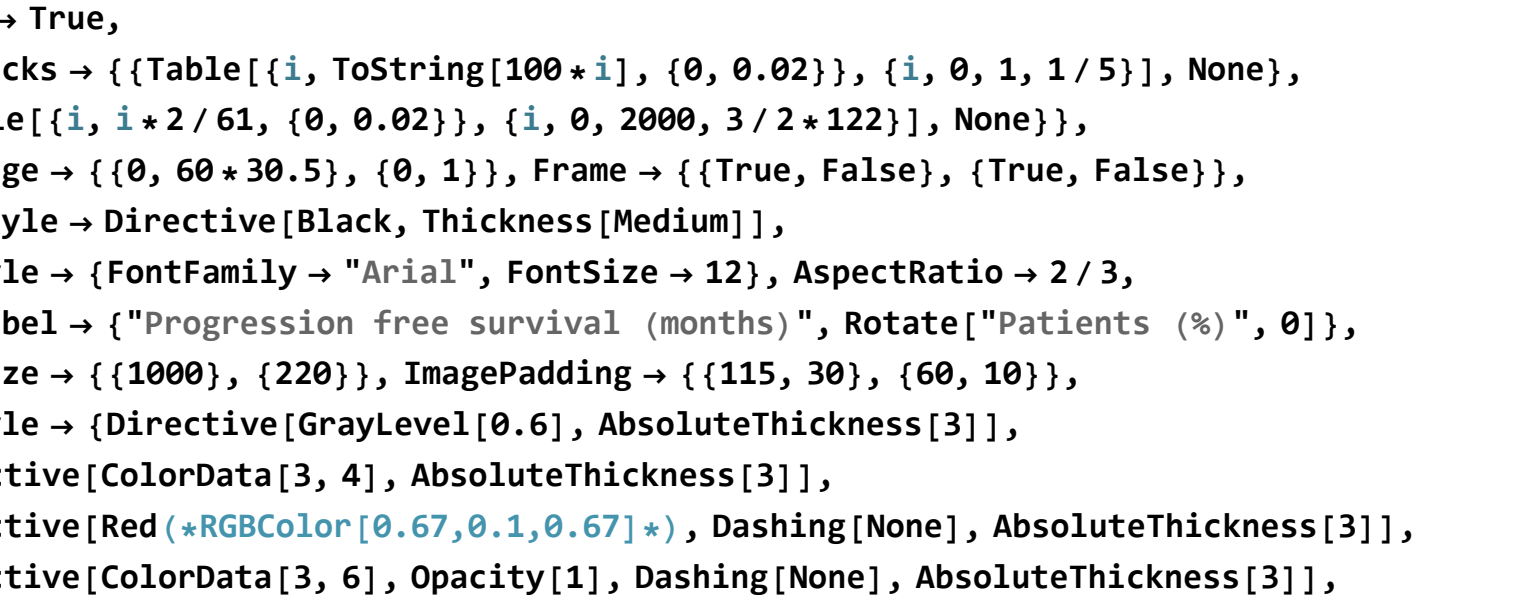
```
 parameters: response correlation between drugs ("Correlation" parameter) ,
and an additive-or-synergistic effect that manifests as the drugs having greater
  potency than monotherapy ("DoseChange" parameter) *)
SimulatePopulationMixed[Correlation_, DoseChange_] := Module[{},

  KI1Table =
   Table[RandomVariate[NormalDistribution[MedianLogDrugSensitivity,
      StandardDeviationInDrugSensitivity]], {1000}];
  KI2Table =
   Table[RandomVariate[NormalDistribution[MedianLogDrugSensitivity,
      StandardDeviationInDrugSensitivity]], {1000}];

  KIJointDistribution = {KI1Table, (Correlation * KI1Table + (1 - Correlation^2) KI2Table)}^T;

  logKI1 = 10^KIJointDistribution[[All, 1]];
  logKI2 = 10^KIJointDistribution[[All, 2]];

  NumberOfTumorsSimulated = 200;
  ProgressionThreshold = 2 * 10^10;

  UntreatedProgressionTimesMixed =
   Parallelize[Table[ProgressionTimeMixedEffects[1000, 1000, DistributionOfGrowthRates[[i]],
      1, ProgressionThreshold], {i, 1, NumberOfTumorsSimulated}]];
  MonotherapyProgressionTimesMixed =
   Parallelize[Table[ProgressionTimeMixedEffects[logKI1[[i]], 1000, DistributionOfGrowthRates[[i]],
      1, ProgressionThreshold], {i, 1, NumberOfTumorsSimulated}]];
  CombinationProgressionTimesMixed =
   Parallelize[Table[ProgressionTimeMixedEffects[logKI1[[i]], logKI2[[i]],
      DistributionOfGrowthRates[[i]], DoseChange, ProgressionThreshold],
     {i, 1, NumberOfTumorsSimulated}]];

  SurvivalPlotMixed = ListPlot[{
     Table[{x, SurvivalFunction[EmpiricalDistribution[UntreatedProgressionTimesMixed]][x]},
      {x, 0, 5 * 365, 2}],
     Table[{x, SurvivalFunction[EmpiricalDistribution[MonotherapyProgressionTimesMixed]][x]},
      {x, 0, 5 * 365, 2}],
     Table[{x, SurvivalFunction[EmpiricalDistribution[CombinationProgressionTimesMixed]][x]},
      {x, 0, 5 * 365, 2}]
    },
     Joined → True, FrameTicks → {Table[{i, i * 2 / 61, {0.015, 0}}, {i, 0, 2000, 3 / 2 * 122}],
       Table[{i, 100 * i, {0.015, 0}}, {i, 0, 1, 1 / 5}]}, PlotRange → {{0, 36 * 30.5}, {0, 1}},
     Frame → {{True, False}, {True, False}}, FrameStyle → Directive[Black, Thickness[Medium]],
     BaseStyle → {FontFamily → "Arial", FontSize → 12}, AspectRatio → 3 / 4,
     FrameLabel → {"Progression free survival (months)", "Patients (%)"},
     ImageSize → {{250}, {250}}, ImagePadding → {{45, 10}, {45, 10}},
     PlotStyle → {Directive[GrayLevel[0.6], Thickness[0.015]],
       Directive[ColorData[3, 4], Thickness[0.015]],
       Directive[ColorData[3, 6], Thickness[0.015]]}]
 ]

(* simulation with response correlation = 0.0, and no synergy or additivity *)
SimulatePopulationMixed[0, 1]
```




```
(* simulation with strong response correlation (ρ=0.7),
and small benefit of the 'additivity-or-
 synergy' type
  (1.4× dose potency - technically 'sub-
    additive' but nonetheless stronger than monotherapy) *)
SimulatePopulationMixed[0.7, 1.4]
```



```
SynergyVSHeterogeneityComparisonPlot = ListPlot[{
   Table[{x, SurvivalFunction[EmpiricalDistribution[UntreatedProgressionTimes[0]]][x]},
    {x, 0, 5 * 365, 2}],
   Table[{x, SurvivalFunction[EmpiricalDistribution[Monotherapy1ProgressionTimes[0]]][x]},
    {x, 0, 5 * 365, 2}],
   Table[
    {x, SurvivalFunction[EmpiricalDistribution[(* combination with 3× synergy *)
        CombinationProgressionTimesSynergy[3]]][x]}, {x, 0, 5 * 365, 2}],
   Table[
    {x,
     SurvivalFunction[EmpiricalDistribution[
        (* combination with independent action and no correlation *)
        CombinationProgressionTimes[0]]][x]}, {x, 0, 5 * 365, 2}],
   Table[
    {x,
     SurvivalFunction[EmpiricalDistribution[
        (* combination with mixed effects: 1.4× synergy and correlation 0.7 *)
        CombinationProgressionTimesMixed]][x]}, {x, 0, 5 * 365, 2}]
   },
   Joined → True,
   FrameTicks → {{Table[{i, ToString[100 * i], {0, 0.02}}, {i, 0, 1, 1 / 5}], None},
     {Table[{i, i * 2 / 61, {0, 0.02}}, {i, 0, 2000, 3 / 2 * 122}], None}},
   PlotRange → {{0, 60 * 30.5}, {0, 1}}, Frame → {{True, False}, {True, False}},
   FrameStyle → Directive[Black, Thickness[Medium]],
   BaseStyle → {FontFamily → "Arial", FontSize → 12}, AspectRatio → 2 / 3,
   FrameLabel → {"Progression free survival (months)", Rotate["Patients (%)", 0]},
   ImageSize → {{1000}, {220}}, ImagePadding → {{115, 30}, {60, 10}},
   PlotStyle → {Directive[GrayLevel[0.6], AbsoluteThickness[3]],
     Directive[ColorData[3, 4], AbsoluteThickness[3]],
     Directive[Red(*RGBColor[0.67,0.1,0.67]*), Dashing[None], AbsoluteThickness[3]],
     Directive[ColorData[3, 6], Opacity[1], Dashing[None], AbsoluteThickness[3]],
     Directive[ColorData[3, 1], Opacity[1], Dashing[None], AbsoluteThickness[3]]},
   PlotLegends → Placed[{"Untreated", "Monotherapy", "Combination,\nSynergy: 3× higher dose",
      "Combination,\nIndependent drug action (ρ=0.0)",
      "Combination, mixed effects:\nhighly correlated responses (ρ=0.7),\nsub-additive
        (1.4× higher dose)"}, Right]]

Export[NotebookDirectory[] <>
   "Supplementary Figure S7C, progression free survival, mixture of additivity and
partially correlated sensitivity.pdf", %, "PDF"];
```



Next we consider a three-drug combination, consisting of two drugs that are 'synergistic' according to Loewe / Chou-Talalay (behaving together as a 3× dose of one drug), and a third drug that is independent (fully uncorrelated responsiveness):

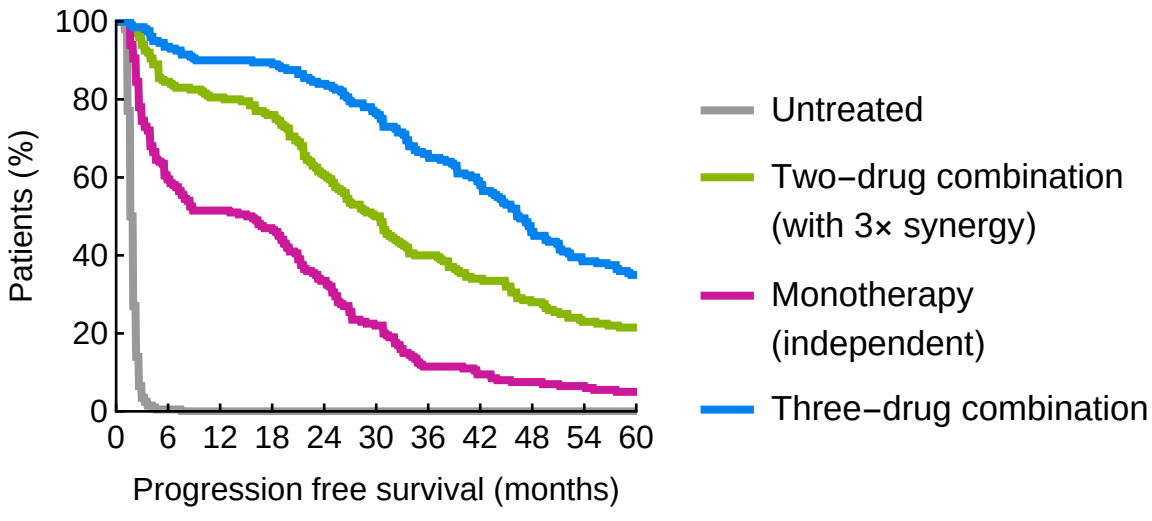
```
DrugDose = 0.2;
```

```
ProgressionTimeThreeDrugs[KI1_, KI2_, growthrate_, ProgressionThreshold_] := Module[{},
  KI1internal = KI1;
  KI2internal = KI2;
  gr = growthrate;
  celllossrate = CellLossRate; (* 80% cell loss rate *)
  dr = gr * celllossrate;
  rl = 10;
  rf1 = 500 * 10^-6;
  rf2 = 500 * 10^-6;
  rfCross = 5 * 10^-6;
  p0 = 10^10;
  d = DrugDose;
  (*synergy, applied ONLY to drug #1 –
   we are saying that "drug 1" in the model is the sum effect of two coadministered
    therapies that act together as one drug at enhanced dose,
  according to the 'synergy' parameter *)
  synergy = 3;
  SimulationDuration = 6; (*years*)

  mysimresultUntreated =
   NDSolve[{p'[t] == gr * p[t] - dr * p[t], p[0] == p0}, p, {t, 0, SimulationDuration * 365}][[1]];
  mysimresultS =
   NDSolve[
     {p'[t] ==
       gr * p[t] * DrugTreatmentOverTime[t, LogDrugInhibitionModel /.
            {LogDrug → Log[10, d],
             LogKI → Min[{Log[10, KI1internal / synergy], Log[10, KI2internal]}], NH → 1}] -
        dr * p[t], p[0] == p0}, p, {t, 0, SimulationDuration * 365}][[1]];
  mysimresultR1 =
   NDSolve[
     {p'[t] ==
       gr * p[t] * DrugTreatmentOverTime[t, LogDrugInhibitionModel /.
            {LogDrug → Log[10, d],
             LogKI → Min[{Log[10, KI1internal * rl / synergy], Log[10, KI2internal]}], NH → 1}] -
        dr * p[t], p[0] == p0 * rf1}, p, {t, 0, SimulationDuration * 365}][[1]];
  mysimresultR2 =
   NDSolve[
     {p'[t] ==
       gr * p[t] * DrugTreatmentOverTime[t, LogDrugInhibitionModel /.
            {LogDrug → Log[10, d],
             LogKI → Min[{Log[10, KI1internal / synergy], Log[10, KI2internal * rl]}], NH → 1}] -
        dr * p[t], p[0] == p0 * rf2}, p, {t, 0, SimulationDuration * 365}][[1]];
  mysimresultRR =
   NDSolve[
     {p'[t] ==
       gr * p[t] * DrugTreatmentOverTime[t, LogDrugInhibitionModel /.
            {LogDrug → Log[10, d],
             LogKI → Min[{Log[10, KI1internal * rl / synergy], Log[10, KI2internal * rl]}], NH → 1}] -
        dr * p[t], p[0] == p0 * rfCross}, p, {t, 0, SimulationDuration * 365}][[1]];

  PopulationOverTimeTable =
   Table[{t, (p[t] /. mysimresultS) + (p[t] /. mysimresultR1) + (p[t] /. mysimresultR2) +
      (p[t] /. mysimresultRR)}, {t, 0, (SimulationDuration - 0.5) * 365, 10}];

  If[PopulationOverTimeTable[[-1, 2]] < ProgressionThreshold,
   Return[(SimulationDuration + 1) * 365]];
```

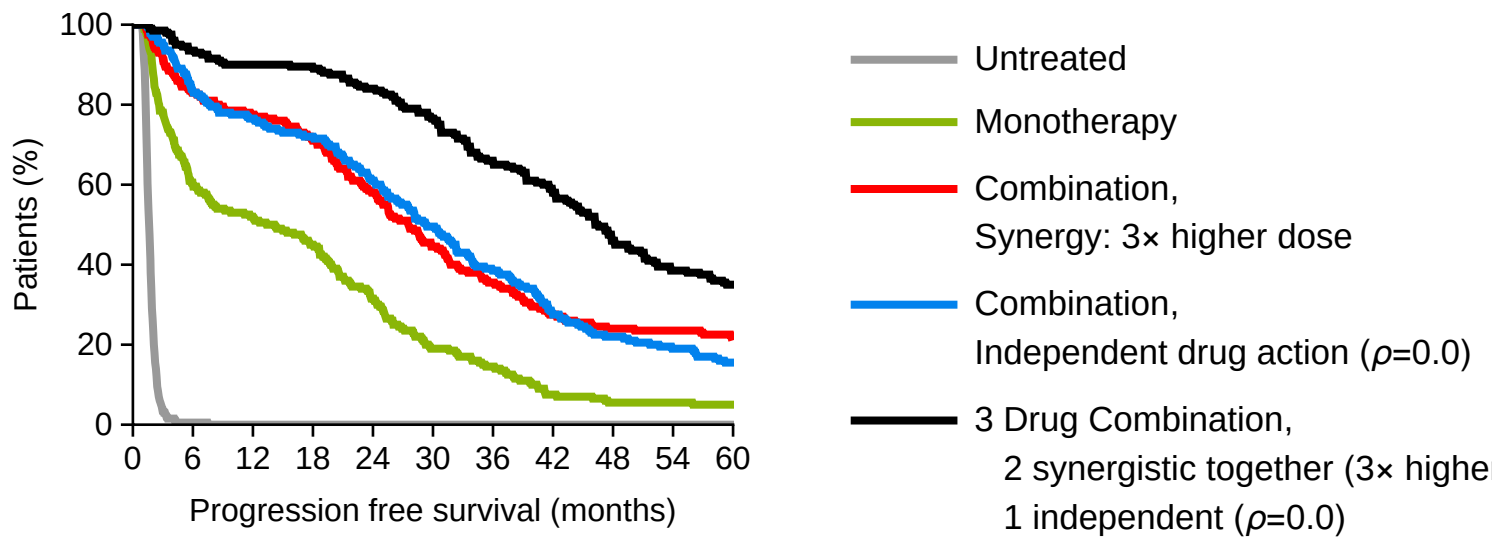
```
 Select[PopulationOverTimeTable, #[[2]] > ProgressionThreshold &][[1, 1]]
]
```

```
SimulatePopulationThreeDrugs[Correlation_] := Module[{},

  KI1Table =
   Table[RandomVariate[NormalDistribution[MedianLogDrugSensitivity,
      StandardDeviationInDrugSensitivity]], {1000}];
  KI2Table =
   Table[RandomVariate[NormalDistribution[MedianLogDrugSensitivity,
      StandardDeviationInDrugSensitivity]], {1000}];

  KIJointDistribution = {KI1Table, (Correlation * KI1Table + (1 - Correlation^2) KI2Table)}^T;

  logKI1 = 10^KIJointDistribution[[All, 1]];
  logKI2 = 10^KIJointDistribution[[All, 2]];

  NumberOfTumorsSimulated = 200;
  ProgressionThreshold = 2 * 10^10;

  UntreatedProgressionTimesThreeDrugs =
   Parallelize[Table[ProgressionTimeThreeDrugs[1000, 1000, DistributionOfGrowthRates[[i]],
      ProgressionThreshold], {i, 1, NumberOfTumorsSimulated}]];
 TwoDrugCombinationProgressionTimesThreeDrugs =
  Parallelize[Table[ProgressionTimeThreeDrugs[logKI1[[i]], 1000, DistributionOfGrowthRates[[i]],
     ProgressionThreshold], {i, 1, NumberOfTumorsSimulated}]];
 IndependentDrugProgressionTimesThreeDrugs =
  Parallelize[Table[ProgressionTimeThreeDrugs[1000, logKI2[[i]], DistributionOfGrowthRates[[i]],
     ProgressionThreshold], {i, 1, NumberOfTumorsSimulated}]];
 CombinationProgressionTimesThreeDrugs =
  Parallelize[Table[ProgressionTimeThreeDrugs[logKI1[[i]], logKI2[[i]],
     DistributionOfGrowthRates[[i]], ProgressionThreshold], {i, 1, NumberOfTumorsSimulated}]];

 SurvivalPlotThreeDrugs = ListPlot[{
    Table[{x, SurvivalFunction[EmpiricalDistribution[UntreatedProgressionTimesThreeDrugs]][x]},
     {x, 0, 5 * 365, 2}],
    Table[
     {x, SurvivalFunction[EmpiricalDistribution[TwoDrugCombinationProgressionTimesThreeDrugs]][
       x]}, {x, 0, 5 * 365, 2}],
    Table[
     {x, SurvivalFunction[EmpiricalDistribution[IndependentDrugProgressionTimesThreeDrugs]][
       x]}, {x, 0, 5 * 365, 2}],
     Table[{x, SurvivalFunction[EmpiricalDistribution[CombinationProgressionTimesThreeDrugs]][
        x]}, {x, 0, 5 * 365, 2}]
    },
    Joined → True, FrameTicks → {Table[{i, i * 2 / 61, {0.015, 0}}, {i, 0, 2000, 3 / 2 * 122}],
      Table[{i, 100 * i, {0.015, 0}}, {i, 0, 1, 1 / 5}]}, PlotRange → {{0, 60 * 30.5}, {0, 1}},
    Frame → {{True, False}, {True, False}}, FrameStyle → Directive[Black, Thickness[Medium]],
    BaseStyle → {FontFamily → "Arial", FontSize → 12}, AspectRatio → 3 / 4,
    FrameLabel → {"Progression free survival (months)", "Patients (%)"},
    ImageSize → {{250}, {250}}, ImagePadding → {{45, 10}, {45, 10}},
    PlotStyle → {Directive[GrayLevel[0.6], AbsoluteThickness[3]],
      Directive[ColorData[3, 4], AbsoluteThickness[3]],
      Directive[RGBColor[0.8, 0.1, 0.6], AbsoluteThickness[3]],
      Directive[ColorData[3, 6], AbsoluteThickness[3]]},
    PlotLegends → {"Untreated", "Two-drug combination\n(with 3× synergy)",
      "Monotherapy\n(independent)", "Three-drug combination"}]
]
```

SimulatePopulationThreeDrugs[0]



```
SynergyVSHeterogeneityComparisonPlot = ListPlot[{
   Table[{x, SurvivalFunction[EmpiricalDistribution[UntreatedProgressionTimes[0]]][x]},
    {x, 0, 5 * 365, 2}],
   Table[{x, SurvivalFunction[EmpiricalDistribution[Monotherapy1ProgressionTimes[0]]][x]},
    {x, 0, 5 * 365, 2}],
   Table[{x, SurvivalFunction[EmpiricalDistribution[CombinationProgressionTimesSynergy[3]]][
      x]}, {x, 0, 5 * 365, 2}],
   Table[{x, SurvivalFunction[EmpiricalDistribution[CombinationProgressionTimes[0]]][x]},
    {x, 0, 5 * 365, 2}],
   Table[{x, SurvivalFunction[EmpiricalDistribution[CombinationProgressionTimesThreeDrugs]][x]},
    {x, 0, 5 * 365, 2}]
  },
  Joined → True,
  FrameTicks → {{Table[{i, ToString[100 * i], {0, 0.02}}, {i, 0, 1, 1 / 5}], None},
    {Table[{i, i * 2 / 61, {0, 0.02}}, {i, 0, 2000, 3 / 2 * 122}], None}},
  PlotRange → {{0, 2 * 61 * 15}, {0, 1}}, Frame → {{True, False}, {True, False}},
  FrameStyle → Directive[Black, Thickness[Medium]],
  BaseStyle → {FontFamily → "Arial", FontSize → 12}, AspectRatio → 2 / 3,
  FrameLabel → {"Progression free survival (months)", Rotate["Patients (%)", 0]},
  ImageSize → {{1000}, {220}}, ImagePadding → {{115, 30}, {60, 10}},
  PlotStyle → {Directive[GrayLevel[0.6], AbsoluteThickness[3]],
    Directive[ColorData[3, 4], AbsoluteThickness[3]],
    Directive[Red(*RGBColor[0.67,0.1,0.67]*), Dashing[None], AbsoluteThickness[3]],
    Directive[ColorData[3, 6], Opacity[1], Dashing[None], AbsoluteThickness[3]],
    Directive[Black, Opacity[1], Dashing[None], AbsoluteThickness[3]]},
  PlotLegends → Placed[{"Untreated", "Monotherapy", "Combination,\nSynergy: 3× higher dose",
     "Combination,\nIndependent drug action (ρ=0.0)",
     "3 Drug Combination,\n   2 synergistic together (3× higher dose),\n   1
       independent (ρ=0.0)"}, Right]]

Export[NotebookDirectory[] <>
   "Supplementary Figure S7C, progression free survival, 3 drug mix of synergy and
     independence.pdf", %, "PDF"];
```



Modeling dose-reduction or antagonistic interactions in combination therapy, as compared to precision monotherapy.

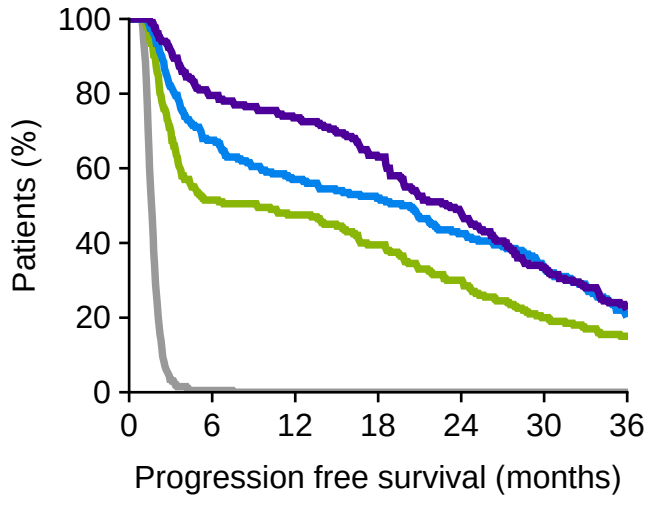
A combination that must be administered with lower doses of each agent is functionally equivalent to a combination without dose-reduction but with an antagonistic drug interactios that causes each drug to act as being equivalent to a lower dose.
This scenario can be simulated by adapting the previously defined code for ‘mixed effects’

(benefit both from uncorrelated responses between drugs, and benefit from synergy), but setting the 'DoseChange' parameter to be smaller than 1.0, indicated that each drug acts as equivalent to a smaller dose than monotherapy.

Precision monotherapy, meaning that each patient receives the one drug to which their tumor is most sensitive, is simulated as monotherapy but with the drug sensitivity parameter (KI) set to the smaller of the two independently sampled KI parameters (one KI value for each drug).

```
SimulatePopulationPrecisionMonotherapy[Correlation_, DoseChange_] := Module[{},

  KI1Table =
   Table[RandomVariate[NormalDistribution[MedianLogDrugSensitivity,
      StandardDeviationInDrugSensitivity]], {1000}];

  KI2Table =
   Table[RandomVariate[NormalDistribution[MedianLogDrugSensitivity,
      StandardDeviationInDrugSensitivity]], {1000}];

  KIJointDistribution = {KI1Table, (Correlation * KI1Table + (1 - Correlation^2) KI2Table)}^T;

  logKI1 = 10^KIJointDistribution[[All, 1]];
  logKI2 = 10^KIJointDistribution[[All, 2]];

  NumberOfTumorsSimulated = 200;
  ProgressionThreshold = 2 * 10^10;

  UntreatedProgressionTimesPrecision =
   Parallelize[Table[ProgressionTimeMixedEffects[1000, 1000, DistributionOfGrowthRates[[i]],
      1, ProgressionThreshold], {i, 1, NumberOfTumorsSimulated}]];
  MonotherapyProgressionTimesPrecision =
   Parallelize[Table[ProgressionTimeMixedEffects[logKI1[[i]], 1000, DistributionOfGrowthRates[[i]],
      1, ProgressionThreshold], {i, 1, NumberOfTumorsSimulated}]];
  CombinationProgressionTimesPrecision =
   Parallelize[Table[ProgressionTimeMixedEffects[logKI1[[i]], logKI2[[i]],
      DistributionOfGrowthRates[[i]], DoseChange, ProgressionThreshold],
     {i, 1, NumberOfTumorsSimulated}]];
  PrecisionMonotherapyProgressionTimesPrecision =
   Parallelize[Table[ProgressionTimeMixedEffects[Min[{logKI1[[i]], logKI2[[i]]}], 1000,
      DistributionOfGrowthRates[[i]], 1, ProgressionThreshold], {i, 1, NumberOfTumorsSimulated}]];

  SurvivalPlotPrecision = ListPlot[{
     Table[{x, SurvivalFunction[EmpiricalDistribution[UntreatedProgressionTimesPrecision]][x]},
      {x, 0, 3 * 365, 2}],
     Table[{x, SurvivalFunction[EmpiricalDistribution[MonotherapyProgressionTimesPrecision]][
        x]}, {x, 0, 3 * 365, 2}],
     Table[{x, SurvivalFunction[EmpiricalDistribution[CombinationProgressionTimesPrecision]][
        x]}, {x, 0, 3 * 365, 2}],
      Table[
       {x,
        SurvivalFunction[EmpiricalDistribution[PrecisionMonotherapyProgressionTimesPrecision]][
         x]}, {x, 0, 3 * 365, 2}]
     },
    Joined → True, FrameTicks → {{Table[{i, ToString[100 * i], {0, 0.02}}, {i, 0, 1, 1 / 5}], None},
      {Table[{i, i * 2 / 61, {0, 0.02}}, {i, 0, 2000, 3 / 2 * 122}], None}},
    PlotRange → {{0, 36 * 30.5}, {0, 1}}, Frame → {{True, False}, {True, False}},
    FrameStyle → Directive[Black, Thickness[Medium]],
    BaseStyle → {FontFamily → "Arial", FontSize → 12}, AspectRatio → 3 / 4,
    FrameLabel → {"Progression free survival (months)", "Patients (%)"},
    ImageSize → {{1000}, {200}}, ImagePadding → {{60, 10}, {50, 10}},
    PlotStyle → {Directive[GrayLevel[0.6], Thickness[0.015]],
      Directive[ColorData[3, 4], Thickness[0.015]], Directive[ColorData[3, 6], Thickness[0.015]],
      Directive[RGBColor[0.3, 0, 0.6], Thickness[0.015]]}]
]
```

```
(* simulating the case of dose reduction to 66% of monotherapy dose,
in comparison to precision monotherapy *)
SimulatePopulationPrecisionMonotherapy[0.0, 0.66]
```




```
Export[NotebookDirectory[] <>
   "Figure 6F, Precision monotherapy and dose reduced combination.pdf",
  SurvivalPlotPrecision, "PDF"];
```

Exploring the effect of variable drug potency (effective dose) and variable cross-resistance

We have so far examined combinations of two equally effective monotherapies. More commonly, clinical trials combine the best available treatment with other treatments that, by themselves, are less effective.
We have also mostly examined the most simple scenario of fully uncorrelated responses. But tumors can of course be multi-drug resistant, and drugs with similar mechanisms can be expected to show activity on an overlapping population, that is, having correlated responses. Analysis of human clinical trials (Figures 1, 4) show that the observed effects of drugs in combination are most often consistent with partially correlated responses, and analysis of PDX drug trial data (Figure 2) also shows that drugs most often have partially correlated responses.

We therefore adapt the previously described models to vary the relative potency of a second drug and to vary correlation in sensitivity to the two drugs.
We also assume that the more correlated is sensitivity, the more overlapping are resistant sub-populations.
Two drugs that share a common molecular target (for example, trastuzumab and pertuzumab; or methotrexate and pralatrexate) are expected to have very highly correlated responses (tumors resistant to one of these drugs are likely resistant to the other drug), and similarly, tumor sub-populations with mutations that increase their resistance to one drug are also likely to be more resistant to the second drug.
Thus, the degree of cross-resistance scales with correlation in responsiveness, with a minimum of 1% cross-resistance as has been used so far.

```
SurvivalTimeVariableDoseVariableCrossResistance[KI1_, KI2_, growthrate_,
  progressionthreshold_, FirstDrugDose_, SecondDrugDose_, CrossResistance_] := Module[{},
  (* potency of each drug needs a non-zero minimum to be enforced *)
  MinimumDrugPotency = 10^-3;

  (* changes in relative dose of the first and second drug are most easily implemented
   by scaling KI for the appropriate drug *)
  KI1internal = KI1 / Max[{FirstDrugDose, MinimumDrugPotency}];
  KI2internal = KI2 / Max[{SecondDrugDose, MinimumDrugPotency}];
```

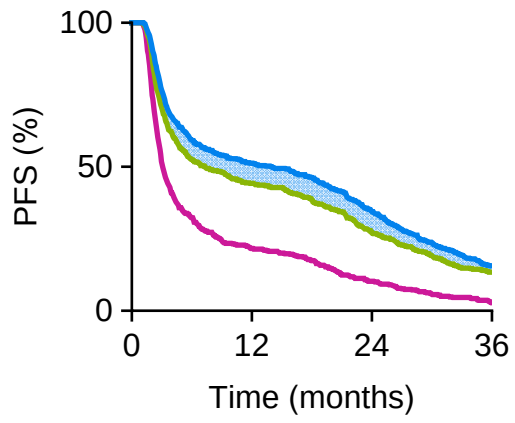
```
(*cell division rate *)
gr = growthrate;
(*spontaneous cell loss rate*)
celllossrate = CellLossRate; (* previously defined as 80% *)
(* death rate = growth rate × cell loss factor *)
dr = gr * celllossrate;
(* rl = 'resistance level', being the fold-change in KI in drug resistant sub-populations *)
rl = 10;

(* pre-existing resistance frequencies *)
(* resistance to drug 1 *) rf1 = 500 * 10^-6 * (1 - CrossResistance);
(* resistance to drug 2 *) rf2 = 500 * 10^-6 * (1 - CrossResistance);
(* resistance to both drugs *) rfCross = Max[{5 * 10^-6, 500 * 10^-6 * CrossResistance}];

(* initial population size *)
p0 = 10^10;
(*drug dose, previously defined *)
d = DrugDose;
(* duration of numerical simulation *)
SimulationDuration = 6; (*years*)

(* simulating an untreated tumor *)
mysimresultUntreated =
 NDSolve[{p'[t] == gr * p[t] - dr * p[t], p[0] == p0}, p, {t, 0, SimulationDuration * 365}][[1]];
(* simulating growth of tumor cells with baseline drug sensitivity *)
mysimresultS =
 NDSolve[
   {p'[t] ==
     gr * p[t] * DrugTreatmentOverTime[t, LogDrugInhibitionModel /.
          {LogDrug → Log[10, d], LogKI → Min[{Log[10, KI1internal], Log[10, KI2internal]}],
           NH → 1}] - dr * p[t], p[0] == p0}, p, {t, 0, SimulationDuration * 365}][[1]];
(* simulating growth of subpopulation with resistance to drug 1 *)
mysimresultR1 =
 NDSolve[
   {p'[t] ==
     gr * p[t] * DrugTreatmentOverTime[t, LogDrugInhibitionModel /.
          {LogDrug → Log[10, d], LogKI → Min[{Log[10, KI1internal * rl], Log[10, KI2internal]}],
           NH → 1}] - dr * p[t], p[0] == p0 * rf1}, p, {t, 0, SimulationDuration * 365}][[1]];
(* simulating growth of subpopulation with resistance to drug 2 *)
mysimresultR2 =
 NDSolve[
   {p'[t] ==
     gr * p[t] * DrugTreatmentOverTime[t, LogDrugInhibitionModel /.
          {LogDrug → Log[10, d], LogKI → Min[{Log[10, KI1internal], Log[10, KI2internal * rl]}],
           NH → 1}] - dr * p[t], p[0] == p0 * rf2}, p, {t, 0, SimulationDuration * 365}][[1]];
(* simulating growth of subpopulation with resistance to both drugs *)
mysimresultRR =
 NDSolve[
   {p'[t] ==
     gr * p[t] * DrugTreatmentOverTime[t, LogDrugInhibitionModel /.
          {LogDrug → Log[10, d],
           LogKI → Min[{Log[10, KI1internal * rl], Log[10, KI2internal * rl]}], NH → 1}] - dr * p[t],
    p[0] == p0 * rfCross}, p, {t, 0, SimulationDuration * 365}][[1]];

(* Summing up the total population size at each time; sum of populations S, R1,
R2, RR. Table has an entry at every 2 days. *)
PopulationOverTimeTable =
```

```
    Table[{t, (p[t] /. mysimresultS) + (p[t] /. mysimresultR1) + (p[t] /. mysimresultR2) +
       (p[t] /. mysimresultRR)}, {t, 0, (SimulationDuration - 0.1) * 365, 2}];

  (* If progression threshold is not reached within the duration of the simulation,
  return a number larger than the duration of simulation
   (this can be used to identify such events) *)
  If[PopulationOverTimeTable[[-1, 2]] < ProgressionThreshold,
   Return[(SimulationDuration + 1) * 365]];

  (* final output: the earliest time at which the total population size surpasses
     the 'progression threshold', taken in this file to be twice the initial population,
  though it could be varied *)
  Select[PopulationOverTimeTable, #[[2]] > ProgressionThreshold &][[1, 1]]

 ]

SimulatePopulationVariableDoseVariableCrossResistance[Correlation_, FirstDrugDose_,
  SecondDrugDose_] := Module[{X1, X2},
KI1Table =
 Table[RandomVariate[NormalDistribution[MedianLogDrugSensitivity,
    StandardDeviationInDrugSensitivity]], {1000}];
KI2Table =
 Table[RandomVariate[NormalDistribution[MedianLogDrugSensitivity,
    StandardDeviationInDrugSensitivity]], {1000}];

KIJointDistribution = {KI1Table, (Correlation * KI1Table + (1 - Correlation^2) KI2Table)}^T;

logKI1 = 10^KIJointDistribution[[All, 1]];
logKI2 = 10^KIJointDistribution[[All, 2]];

NumberOfTumorsSimulated = 500;
ProgressionThreshold = 2 * 10^10;

UntreatedSurvivalTimes[Correlation, SecondDrugDose] =
 Parallelize[Table[SurvivalTimeVariableDoseVariableCrossResistance[1000, 1000,
    DistributionOfGrowthRates[[i]], ProgressionThreshold, FirstDrugDose, SecondDrugDose,
    Correlation], {i, 1, NumberOfTumorsSimulated}]];
Monotherapy1SurvivalTimes[Correlation, SecondDrugDose] =
 Parallelize[Table[SurvivalTimeVariableDoseVariableCrossResistance[logKI1[[i]],
    1000, DistributionOfGrowthRates[[i]], ProgressionThreshold, FirstDrugDose,
    SecondDrugDose, Correlation], {i, 1, NumberOfTumorsSimulated}]];
Monotherapy2SurvivalTimes[Correlation, SecondDrugDose] =
 Parallelize[Table[SurvivalTimeVariableDoseVariableCrossResistance[1000, logKI2[[i]],
    DistributionOfGrowthRates[[i]], ProgressionThreshold, FirstDrugDose, SecondDrugDose,
    Correlation], {i, 1, NumberOfTumorsSimulated}]];
CombinationSurvivalTimes[Correlation, SecondDrugDose] =
 Parallelize[Table[SurvivalTimeVariableDoseVariableCrossResistance[logKI1[[i]],
    logKI2[[i]], DistributionOfGrowthRates[[i]], ProgressionThreshold, FirstDrugDose,
    SecondDrugDose, Correlation], {i, 1, NumberOfTumorsSimulated}]];

SurvivalPlotVariableDoseVariableCrossResistance[Correlation, SecondDrugDose] = ListPlot[{
   Table[
    {x,
     SurvivalFunction[EmpiricalDistribution[Monotherapy2SurvivalTimes[Correlation,
         SecondDrugDose]]][x]}, {x, 0, 5 * 365, 2}],
   Table[
    {x,
```

```
    SurvivalFunction[EmpiricalDistribution[Monotherapy1SurvivalTimes[Correlation,
        SecondDrugDose]]][x]}, {x, 0, 5 * 365, 2}],
 Table[
  {x,
   SurvivalFunction[EmpiricalDistribution[CombinationSurvivalTimes[Correlation,
        SecondDrugDose]]][x]}, {x, 0, 5 * 365, 2}]
},
Joined → True, FrameTicks → {Table[{i, i * 2 / 61, {0, 0.03}}, {i, 0, 2000, 6 / 2 * 122}],
  Table[{i, 100 * i, {0, 0.03}}, {i, 0, 1, 1 / 2}]}, PlotRange → {{0, 36 * 30.5}, {0, 1}},
Frame → {{True, False}, {True, False}}, FrameStyle → Directive[Black, Thickness[Medium]],
BaseStyle → {FontFamily → "Arial", FontSize → 12}, AspectRatio → 4 / 5,
FrameLabel → {"Time (months)", "PFS (%)"}, ImageSize → {{200}, {200}},
ImagePadding → {{55, 10}, {45, 10}},
PlotStyle → {Directive[RGBColor[0.8, 0.1, 0.6], AbsoluteThickness[2]],
  Directive[ColorData[3, 4], AbsoluteThickness[2]],
  Directive[ColorData[3, 6], AbsoluteThickness[2]]}, Filling → {2 → {3}},
FillingStyle → Directive[ColorData[3, 6], Opacity[0.35]]]

]
```

Executing one instance:

```
correlation = 0.5;
firstdrugpotency = 1;
seconddrugpotency = 0.5;
SimulatePopulationVariableDoseVariableCrossResistance[correlation, firstdrugpotency,
 seconddrugpotency]
```



Plotting dependence of median PFS on correlation (this step is time-consuming)

```
(* executing the simulation across a range of correlation values,
from 0 to 1. The results will be stored in the terms '
 Monotherapy1SurvivalTimes[correlation,SecondDrugDose]' and '
 CombinationSurvivalTimes[Correlation,SecondDrugDose]' for the specified values
 of 'correlation' and 'SecondDrugDose'. *)
CorrelationStepSize = 0.025;
Do[SimulatePopulationVariableDoseVariableCrossResistance[correlation, 1, 1],
 {correlation, 0, 1, CorrelationStepSize}]

(* taking the median PFS with monotherapy in these simulations,
computed from the average of the many simulations. Monotherapy efficacy does not
 depend on correlations, so the simulation at different correlations serve as simple
 repeats of the monotherapy efficacy *)
MedianPFSofMonotherapy =
 Mean[Table[Median[Monotherapy1SurvivalTimes[correlation, 1]],
    {correlation, 0, 1, CorrelationStepSize}]] // N
```

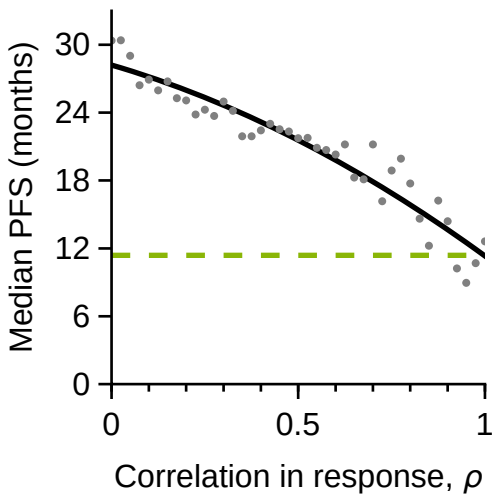
```
347.171
```

```
(* fitting a linear-plus-sigmoidal curve to the relationship between response
  correlation and PFS. We attach no importance to the shape of the function -
 it is merely a line to show the trend *)
nh = 2;
model = b - h * (x + 1)^nh / ((x + 1)^nh + k^nh) - m * x;
sigmoidfit =
 NonlinearModelFit[Table[{correlation, Median[CombinationSurvivalTimes[correlation, 1]] / 30.5},
   {correlation, 0, 1, CorrelationStepSize}], model, {{b, 35}, {h, 1}, {k, 1}, {m, 5}}, x]
```

--- NonlinearModelFit: Failed to converge to the requested accuracy or precision within 100 iterations.

$$\text{FittedModel}\left[35.5153 + 4.93093\,x - \frac{5594.71\,(1+x)^2}{764.445 + (1+x)^2}\right]$$

```
ListPlot[{Table[{correlation, Median[CombinationSurvivalTimes[correlation, 1]] / 30.5},
   {correlation, 0, 1, CorrelationStepSize}],
  Table[{correlation, MedianPFSofMonotherapy / 30.5}, {correlation, 0, 1, CorrelationStepSize}],
  Table[{correlation, sigmoidfit[correlation]}, {correlation, 0, 1, CorrelationStepSize}]},
 Joined → {False, True, True},
 PlotStyle → {Directive[GrayLevel[0.5], AbsolutePointSize[3]],
   Directive[Dashing[{0.05, 0.05}], ColorData[3, 4], AbsoluteThickness[2]],
   Directive[Black, AbsoluteThickness[2]]}, Filling → None,
 FillingStyle → Directive[ColorData[3, 6], Opacity[0.3]], PlotRange → {{0, 1.0}, {0, 33}},
 Frame → {{True, True}, {True, False}},
 FrameStyle → {{Directive[Black, Thickness[Medium]], Directive[White, Opacity[0]]},
   {Directive[Black, Thickness[Medium]], Directive[Black, Thickness[Medium]]}},
 Axes → False, BaseStyle → {FontFamily → "Arial", FontSize → 12}, AspectRatio → 1,
 ImageSize → {{1000}, {200}}, ImagePadding → {{50, 40}, {50, 10}},
 FrameTicks → {{Table[{i, i, {0, 0.035}}, {i, 0, 36, 6}], None},
   {Join[Table[{N[i], i, {0, 0.04}}, {i, -1, 1, 1 / 2}],
      Table[{N[i], , {0, 0.02}}, {i, -1, 1, 1 / 10}]] /. {1 / 2 → "0.5", -1 / 2 → "-0.5"}, None}},
 FrameLabel → {"Correlation in response, ρ", "Median PFS (months)"}]

Export[NotebookDirectory[] <> "Simulated PFS as a function of correlation.pdf", %, "PDF"];
```



Generating a matrix of results, over a range of correlation strengths (from 0 to 1 in steps of 0.1) and a range of relative potency of the second drug (also from 0 to 1 in steps of 0.1)

(the first drug's potency is held constant, and the second drug's potency is adjusted from wholly ineffective to having efficacy equal to the first drug)

Note: this step is time consuming (possibly up to 1 hour)

```
MatrixOfPFStimes1 =
  Table[SimulatePopulationVariableDoseVariableCrossResistance[correlation, 1, relativepotency],
   {correlation, 0.0, 1.0, 0.1}, {relativepotency, 0.0, 1.0, 0.1}];

(* Relative risk is calculated according to the Cox Proportional Hazards Model,
with 'censoring' of any simulated patients that remains progression-
 free by 3
  years. (Note, this does not remove them from analysis,
    but rather caps their recorded survival time at 3 years without enforcing '
     progression' at 3 years) *)
RelativeRisk[correlation_, relativepotency_] := Module[{},
YearToCensorAt = 3;

(* Monotherapy 1 outcomes shorter than 3 years *)
M1below3yr = Select[Sort[Monotherapy1SurvivalTimes[correlation, relativepotency]],
  # <= YearToCensorAt * 365 &];
(* Monotherapy 1 outcomes longer than 3 years *)
M1above3yr = Map[Min[{#, YearToCensorAt * 365}] &,
  Select[Sort[Monotherapy1SurvivalTimes[correlation, relativepotency]],
   # > YearToCensorAt * 365 &]];
(* Combination outcomes shorter than 3 years *)
Cbelow3yr = Select[Sort[CombinationSurvivalTimes[correlation, relativepotency]],
  # <= YearToCensorAt * 365 &];
(* Combination outcomes longer than 3 years *)
Cabove3yr = Map[Min[{#, YearToCensorAt * 365}] &,
  Select[Sort[CombinationSurvivalTimes[correlation, relativepotency]],
   # > YearToCensorAt * 365 &]];

(* Table of 'event data'. See Mathematica help file on CoxModelFit for demonstration
 of syntax. *)
myeventdata = EventData[Join[M1below3yr, M1above3yr, Cbelow3yr, Cabove3yr],
   Join[Table[0, {Length[M1below3yr]}], Table[1, {Length[M1above3yr]}],
    Table[0, {Length[Cbelow3yr]}], Table[1, {Length[Cabove3yr]}]]];
 descriptors =
  Join[Table["One drug",
    {Length[Sort[Monotherapy1SurvivalTimes[correlation, relativepotency]]]}],
   Table["Two drugs",
    {Length[Sort[CombinationSurvivalTimes[correlation, relativepotency]]]}]];

 MyModelFit = CoxModelFit[{descriptors, myeventdata}, {treatment}, {treatment},
   NominalVariables → treatment];

 (* result: the relative risk as calculated by Cox Model,
 for combination therapy versus monotherapy *)
 MyModelFit["RelativeRisk"][[1]]
]
```

```
(* turning off error messsage that concerns the program's desire for more significant
 figures *)Off[FindRoot::lstol]
RelativeRiskTable1 = Table[RelativeRisk[correlation, relativepotency],
   {correlation, 0.0, 1.0, 0.1}, {relativepotency, 0.0, 1.0, 0.1}];
Export[NotebookDirectory[] <> "CorrelationVSPotency relative risk table1.csv",
 RelativeRiskTable1, "CSV"]
```

Repeating the simulations, for a total of 5 times, exporting results

Note: this step is time consuming (possibly hours)

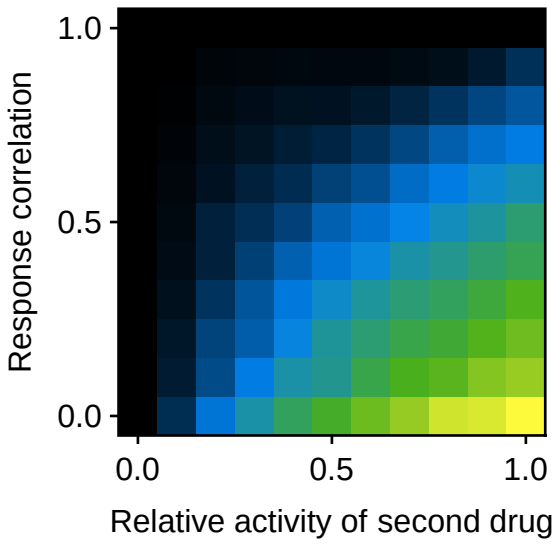
```
MatrixOfPFStimes2 =
  Table[SimulatePopulationVariableDoseVariableCrossResistance[correlation, 1, relativepotency],
   {correlation, 0.0, 1.0, 0.1}, {relativepotency, 0.0, 1.0, 0.1}];
RelativeRiskTable2 = Table[RelativeRisk[correlation, relativepotency],
   {correlation, 0.0, 1.0, 0.1}, {relativepotency, 0.0, 1.0, 0.1}];
Export[NotebookDirectory[] <> "CorrelationVSPotency relative risk table2.csv",
 RelativeRiskTable2, "CSV"]

MatrixOfPFStimes3 =
  Table[SimulatePopulationVariableDoseVariableCrossResistance[correlation, 1, relativepotency],
   {correlation, 0.0, 1.0, 0.1}, {relativepotency, 0.0, 1.0, 0.1}];
RelativeRiskTable3 = Table[RelativeRisk[correlation, relativepotency],
   {correlation, 0.0, 1.0, 0.1}, {relativepotency, 0.0, 1.0, 0.1}];
Export[NotebookDirectory[] <> "CorrelationVSPotency relative risk table3.csv",
 RelativeRiskTable3, "CSV"]

MatrixOfPFStimes4 =
  Table[SimulatePopulationVariableDoseVariableCrossResistance[correlation, 1, relativepotency],
   {correlation, 0.0, 1.0, 0.1}, {relativepotency, 0.0, 1.0, 0.1}];
RelativeRiskTable4 = Table[RelativeRisk[correlation, relativepotency],
   {correlation, 0.0, 1.0, 0.1}, {relativepotency, 0.0, 1.0, 0.1}];
Export[NotebookDirectory[] <> "CorrelationVSPotency relative risk table4.csv",
 RelativeRiskTable4, "CSV"]

MatrixOfPFStimes5 =
  Table[SimulatePopulationVariableDoseVariableCrossResistance[correlation, 1, relativepotency],
   {correlation, 0.0, 1.0, 0.1}, {relativepotency, 0.0, 1.0, 0.1}];
RelativeRiskTable5 = Table[RelativeRisk[correlation, relativepotency],
   {correlation, 0.0, 1.0, 0.1}, {relativepotency, 0.0, 1.0, 0.1}];
Export[NotebookDirectory[] <> "CorrelationVSPotency relative risk table5.csv",
 RelativeRiskTable5, "CSV"]
```

Importing results and averaging. This reduces random error from different simulations, yielding more precise relative risk values

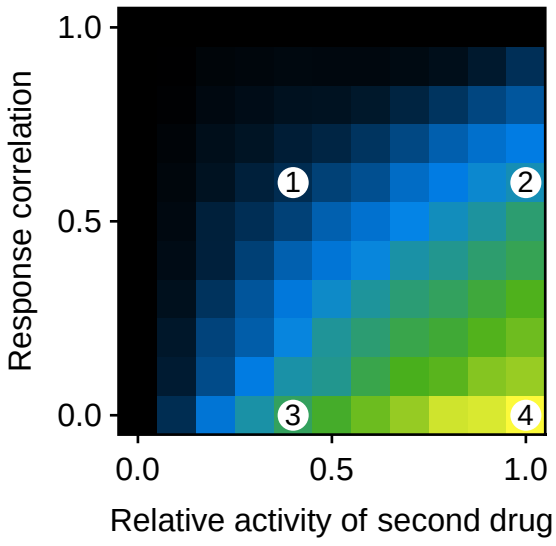
```
RelativeRiskTable1 =
  Import[NotebookDirectory[] <> "CorrelationVSPotency relative risk table1.csv", "CSV"];
RelativeRiskTable2 =
  Import[NotebookDirectory[] <> "CorrelationVSPotency relative risk table2.csv", "CSV"];
RelativeRiskTable3 =
  Import[NotebookDirectory[] <> "CorrelationVSPotency relative risk table3.csv", "CSV"];
RelativeRiskTable4 =
  Import[NotebookDirectory[] <> "CorrelationVSPotency relative risk table4.csv", "CSV"];
RelativeRiskTable5 =
  Import[NotebookDirectory[] <> "CorrelationVSPotency relative risk table5.csv", "CSV"];

AverageRelativeRiskTable =
  Mean[{RelativeRiskTable1, RelativeRiskTable2, RelativeRiskTable3, RelativeRiskTable4,
    RelativeRiskTable5}];
```

heatmap of relative risk

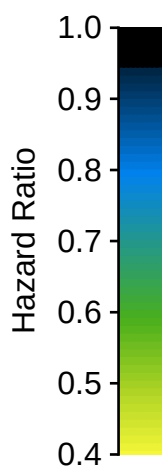
```
(* Defining a custom color scheme *)
Unprotect[ColorData];
ColorData["MyCustom"] =
  Function[x,
   Blend[
    Transpose[{(Range[7] - 1) / 7, {Black, Darker[ColorData[3, 6], 0.5], ColorData[3, 6],
       Blend[{ColorData["AvocadoColors"][0.5], ColorData[3, 6]}, 0.5],
       ColorData["AvocadoColors"][0.5], ColorData["AvocadoColors"][0.75],
       ColorData["AvocadoColors"][1]}}], x]];
Protect[ColorData];

ArrayPlot[Reverse[AverageRelativeRiskTable], ColorFunctionScaling → False,
 ColorFunction → (ColorData["MyCustom"][1 * (1.4 - 1.4 * #)^1] &), PlotRangePadding → None,
 BaseStyle → {FontFamily → "Arial", FontSize → 12},
 FrameStyle → Directive[Black, Thickness[Medium]], ImageSize → {{1000}, {220}},
 ImagePadding → {{50, 10}, {50, 10}},
 FrameTicks → {{{{1, "1.0", {0, 0.02}}, {6, "0.5", {0, 0.02}}, {11, "0.0", {0, 0.02}}}, None},
   {{{1, "0.0", {0, 0.02}}, {6, "0.5", {0, 0.02}}, {11, "1.0", {0, 0.02}}}, None}},
 FrameLabel → {"Response correlation", "Relative activity of second drug"}]
```



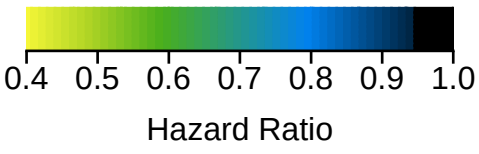
```
CorrelationVsPotencyHeatmap =
 ArrayPlot[Reverse[AverageRelativeRiskTable], ColorFunctionScaling → False,
  ColorFunction → (ColorData["MyCustom"][1 * (1.4 - 1.4 * #)^1] &), PlotRangePadding → None,
  BaseStyle → {FontFamily → "Arial", FontSize → 12},
  FrameStyle → Directive[Black, Thickness[Medium]], ImageSize → {{1000}, {220}},
  ImagePadding → {{50, 10}, {50, 10}},
  FrameTicks → {{{{1, "1.0", {0, 0.02}}, {6, "0.5", {0, 0.02}}, {11, "0.0", {0, 0.02}}}, None},
    {{{1, "0.0", {0, 0.02}}, {6, "0.5", {0, 0.02}}, {11, "1.0", {0, 0.02}}}, None}},
  FrameLabel → {"Response correlation", "Relative activity of second drug"},
  Epilog → {White, Opacity[1], EdgeForm[None], Disk[{4.5, 0.5}, 0.4], Disk[{10.5, 6.5}, 0.4],
    Disk[{4.5, 6.5}, 0.4], Disk[{10.5, 0.5}, 0.4], Black, FontSize → 11,
    Text["1", {4.5, 6.5}, {0, 0}], Text["2", {10.5, 6.5}, {0, 0}], Text["3", {4.5, 0.5}, {0, 0}],
    Text["4", {10.5, 0.5}, {0, 0}]}]

Export[NotebookDirectory[] <> "Figure 6G, Relative risk heatmap.pdf",
  CorrelationVsPotencyHeatmap, "PDF"];
```




```
RelativeRiskColorscale =
 ContourPlot[y, {x, 0, 1}, {y, 0.4, 1}, ColorFunctionScaling → False,
   ColorFunction → (ColorData["MyCustom"][1 * (1.4 - 1.4 * #)^1] &), PlotRangePadding → None,
   ContourStyle → None, Contours → 50, Frame → {{True, False}, {False, False}},
   FrameStyle → Directive[Black, Thickness[Medium]],
   BaseStyle → {FontFamily → "Arial", FontSize → 12},
   FrameTicks → {{Table[{i, NumberForm[i, {2, 1}]}, {0, 0.2}}, {i, 0.4, 1, 0.1}], None},
     {None, None}}, ImageSize → {{1000}, {220}}, ImagePadding → {{50, 10}, {50, 10}},
   AspectRatio → 10, PerformanceGoal → "Speed", FrameLabel → {, "Hazard Ratio"}]
  (* this next part is necessary to remove mesh lines from the exported PDF *) /.
 {EdgeForm[], r_?(MemberQ[{RGBColor, Hue, CMYKColor, GrayLevel}, Head[#]] &), i___} :→
  {EdgeForm[r], r, i}

Export[NotebookDirectory[] <> "Figure 6G, relative risk colorscale.pdf",
  RelativeRiskColorscale, "PDF"];
```

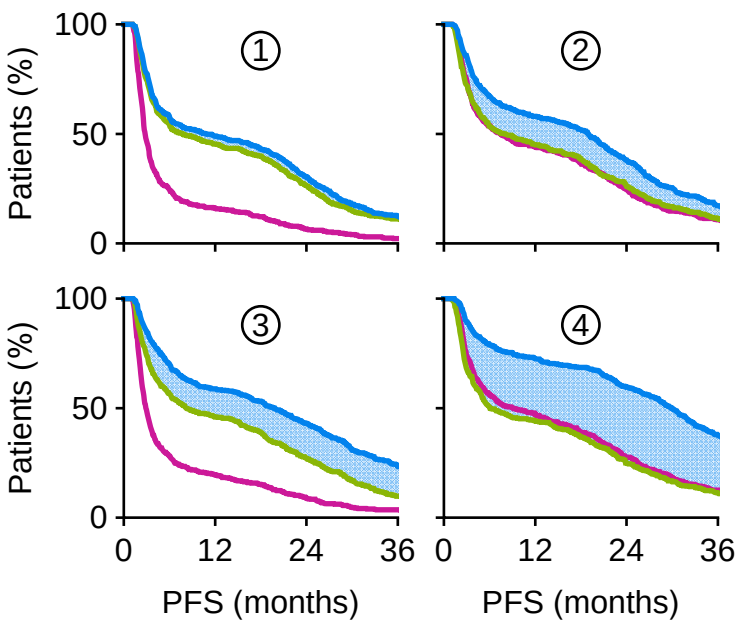


```
RelativeRiskHorizontalColorscale =
 ContourPlot[x, {x, 0.4, 1}, {y, 0, 1}, ColorFunctionScaling → False,
   ColorFunction → (ColorData["MyCustom"][1 * (1.4 - 1.4 * #)^1] &), PlotRangePadding → None,
   ContourStyle → None, Contours → 50, Frame → {{False, False}, {True, False}},
   FrameStyle → Directive[Black, Thickness[Medium]],
   BaseStyle → {FontFamily → "Arial", FontSize → 12},
   FrameTicks → {{None, None}, {Table[{i, NumberForm[i, {2, 1}], {0, 0.03}}, {i, 0.4, 1, 0.1}],
      None}}, ImageSize → {{220}, {220}}, ImagePadding → {{50, 10}, {50, 10}},
   AspectRatio → 1 / 10, FrameLabel → {"Hazard Ratio",}, PerformanceGoal → "Speed"]
   (* this next part is necessary to remove mesh lines from the exported PDF *) /.
  {EdgeForm[], r_?(MemberQ[{RGBColor, Hue, CMYKColor, GrayLevel}, Head[#]] &), i___} :>
   {EdgeForm[r], r, i}

Export[NotebookDirectory[] <> "Figure 6G, relative risk colorscale, horizontal.pdf",
  RelativeRiskHorizontalColorscale, "PDF"];
```




```
Point1Plot = SimulatePopulationVariableDoseVariableCrossResistance[0.6, 1, 0.4];

Point2Plot = SimulatePopulationVariableDoseVariableCrossResistance[0.6, 1, 1.0];

Point3Plot = SimulatePopulationVariableDoseVariableCrossResistance[0.0, 1, 0.4];

Point4Plot = SimulatePopulationVariableDoseVariableCrossResistance[0.0, 1, 1.0];
```

```
(* adjusting icon size *)markerscaling = 160;

CorrelationVsPotencySurvivalCurves = GraphicsGrid[{{
    Show[Point1Plot, FrameTicks → {Table[{i, , {0, 0.03}}, {i, 0, 2000, 6 / 2 * 122}],
       Table[{i, 100 * i, {0, 0.03}}, {i, 0, 1, 1 / 2}]}, FrameLabel → {, "Patients (%)"},
     Epilog →
      Inset[Graphics[{Opacity[1], White,
         EdgeForm[Directive[Opacity[1], Black, AbsoluteThickness[1]]], Disk[{0, 0}, 1],
         Black, FontSize → 12, Text["1", {0, 0}, {0, 0}]}], {18 * 30.5, 0.88}, {0, 0},
       markerscaling]]
    ,
    Show[Point2Plot, FrameTicks → {Table[{i, , {0, 0.03}}, {i, 0, 2000, 6 / 2 * 122}],
       Table[{i, , {0, 0.03}}, {i, 0, 1, 1 / 2}]}, FrameLabel → {"", ""},
     Epilog →
      Inset[Graphics[{Opacity[1], White,
         EdgeForm[Directive[Opacity[1], Black, AbsoluteThickness[1]]], Disk[{0, 0}, 1],
         Black, FontSize → 12, Text["2", {0, 0}, {0, 0}]}], {18 * 30.5, 0.88}, {0, 0},
       markerscaling]]},
    {
     Show[Point3Plot, FrameTicks → {Table[{i, i * 2 / 61, {0, 0.03}}, {i, 0, 2000, 6 / 2 * 122}],
       Table[{i, 100 * i, {0, 0.03}}, {i, 0, 1, 1 / 2}]},
      FrameLabel → {"PFS (months)", "Patients (%)"},
      Epilog →
       Inset[Graphics[{Opacity[1], White,
          EdgeForm[Directive[Opacity[1], Black, AbsoluteThickness[1]]], Disk[{0, 0}, 1],
          Black, FontSize → 12, Text["3", {0, 0}, {0, 0}]}], {18 * 30.5, 0.88}, {0, 0},
        markerscaling]]
     ,
     Show[Point4Plot, FrameTicks → {Table[{i, i * 2 / 61, {0, 0.03}}, {i, 0, 2000, 6 / 2 * 122}],
       Table[{i, , {0, 0.03}}, {i, 0, 1, 1 / 2}]}, FrameLabel → {"PFS (months)",},
      Epilog →
       Inset[Graphics[{Opacity[1], White,
          EdgeForm[Directive[Opacity[1], Black, AbsoluteThickness[1]]], Disk[{0, 0}, 1],
          Black, FontSize → 12, Text["4", {0, 0}, {0, 0}]}], {18 * 30.5, 0.88}, {0, 0},
        markerscaling]]
    }
   }, Spacings → {-60, -40}, ImageSize → {{1000}, {240}}]

Export[NotebookDirectory[] <>
  "Figure 6G, Simulated combinations at different correlation, potency.pdf",
 CorrelationVsPotencySurvivalCurves, "PDF"];
```



End of all figures and simulations in the main text.

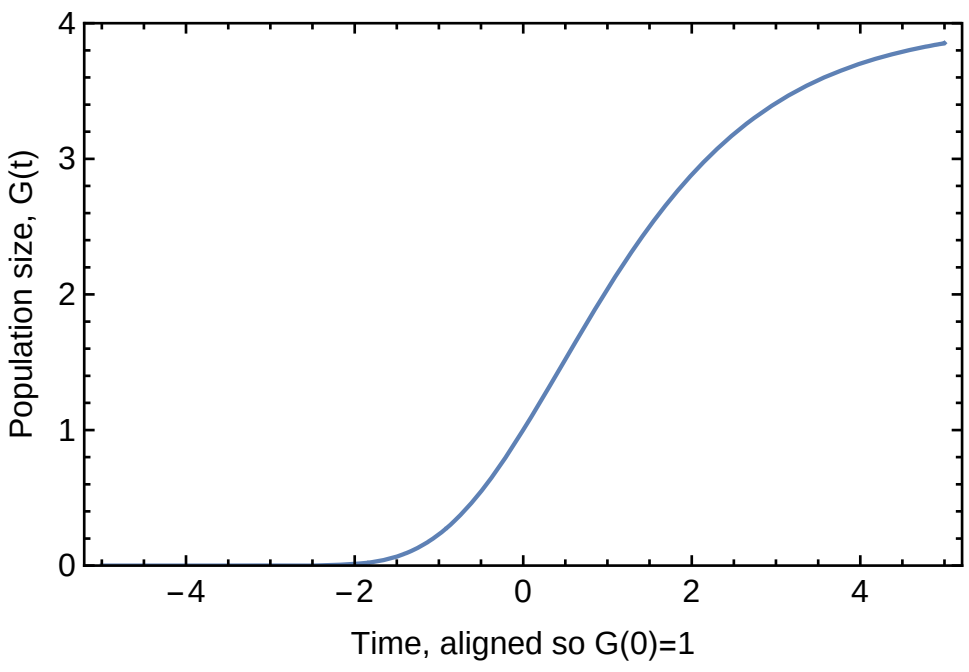
What follows are two explorations of different possible model structures: firstly, Gompertz growth kinetics; secondly, a model of cytotoxic therapy administered in cycles.

The result that independent drug action is sufficient for benefit in a drug combination is demonstrated also in these alternative models.

Exploring the effect of Gompertz growth kinetics

```
Gompertz[t_] := a * Exp[-b * Exp[-c * t]]

(* this set of parameters gives a maximum population size of 4, a population at t=0 of 1,
and a growth rate at t=0 of 1 (we shall adjust this subsequently) *)
GompertzParameters = {a → 4, b → Log[4], c → 1 / Log[4]}

Plot[Gompertz[t] /. GompertzParameters, {t, -5, 5}, PlotRange → {0, 4}, Frame → True,
 FrameLabel → {"Time, aligned so G(0)=1", "Population size, G(t)"}, Axes → False,
 BaseStyle → {FontFamily → "Arial", FontSize → 12},
 FrameStyle → Directive[Black, Thickness[Medium]]]
```

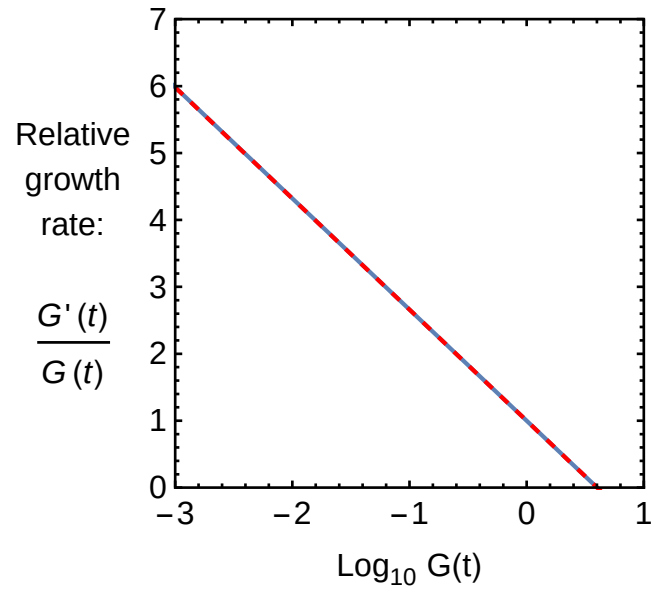
$$\left\{a \to 4,\ b \to \text{Log}[4],\ c \to \frac{1}{\text{Log}[4]}\right\}$$



The characteristic feature of Gompertz growth kinetics is that relative growth rate decreases linearly with respect to the logarithm of the population size, until ultimately the population size reaches an asymptote and growth rate is effectively zero:

```
LinearModelFit[Table[{Log[10, Gompertz[t]], Gompertz'[t] / Gompertz[t]} /. GompertzParameters,
  {t, -4, 4, 0.1}], logp, logp]

Show[
 ParametricPlot[{Log[10, Gompertz[t]], Gompertz'[t] / Gompertz[t]} /. GompertzParameters,
  {t, -4, 4}, PlotRange → {{-3, 1}, {0, 7}}, AspectRatio → 1, Frame → True, Axes → False,
  FrameLabel → {"Log10 G(t)", Rotate["Relative\ngrowth\nrate:\n\n G'(t)/G(t)", -π / 2]},
  BaseStyle → {FontFamily → "Arial", FontSize → 12},
  FrameStyle → Directive[Black, Thickness[Medium]]]
 ,
 Plot[1 - 1.66 * logp, {logp, -3, 1}, PlotStyle → Directive[Red, Dashed]]
 ,
 ImageSize → {{500}, {220}}
]
```

```
FittedModel[ 1. - 1.66096 logp ]
```



We will implement Gompertz kinetics in our previously established model structure by setting growth rate at each time to depend on the population size at that time, according to the relationship plotted above (relative growth rate = $1 - 1.66 \times \log_{10}$(relative population size). (where 'relative population size' is in respect to the population at t=0, being 10^{10} cells in the simulation above.

```
(* function describing drug response over time. Most simply, therapy is continuous,
but this function can be used to describe time-
 dependent therapy (e.g. periodic cycles of treatment) *)
DrugTreatmentOverTime[t_, RelativeGrowthDuringTreatment_] := RelativeGrowthDuringTreatment
(* continuous therapy *)

(* response of growth to drug treatment - a simple Hill function,
hyperbolic in the case of Hill Coefficient NH=1. d = drug dose,
KI = drug sensitivity (IC50) *)
DrugInhibitionModel = 1 / (1 + (drug / KI)^NH);

(* same model as above,
but expressing drug concentration and KI as their logarithms. This makes for easier
  plotting on a log-scale of drug concentration *)
LogDrugInhibitionModel = 1 / (1 + (10^LogDrug / 10^LogKI)^NH);

DrugDose = 0.2;

(* this function takes as its inputs
 KI for drug 1 (KI1),
KI for drug 2 (KI2),
ProgressionThreshold, being how large an increase in tumor volume is our output (2), and,
growthratenumber, which indicates which value from the list of randomly generated
 growth rates is being taken in this particular instance of the simulation. This
 takes integer values indicating a position in the previously generated list '
 DistributionOfGrowthRates'.
*)

ProgressionTimeGompertz[KI1_, KI2_, growthrate_, ProgressionThreshold_] := Module[{},
  KI1internal = KI1;
KI2internal = KI2;
(*cell division rate *)
gr = growthrate;
(*spontaneous cell death rate*)
celllossrate = CellLossRate; (* previously defined as 80% *)
(* death rate = growth rate × cell loss factor *)
dr = gr * celllossrate;
(* rl = 'resistance level', being the fold-change in KI in drug resistant sub-populations *)
rl = 10;

(* pre-existing resistance frequencies *)
(* resistance to drug 1 *) rf1 = 500 * 10^-6;
(* resistance to drug 2 *) rf2 = 500 * 10^-6;
(* resistance to both drugs *) rfCross = 5 * 10^-6;

(* initial population size *)
p0 = 10^10;

(* relative growth rate = RGR ; here is where we implement Gompertzian kinetics *)
```

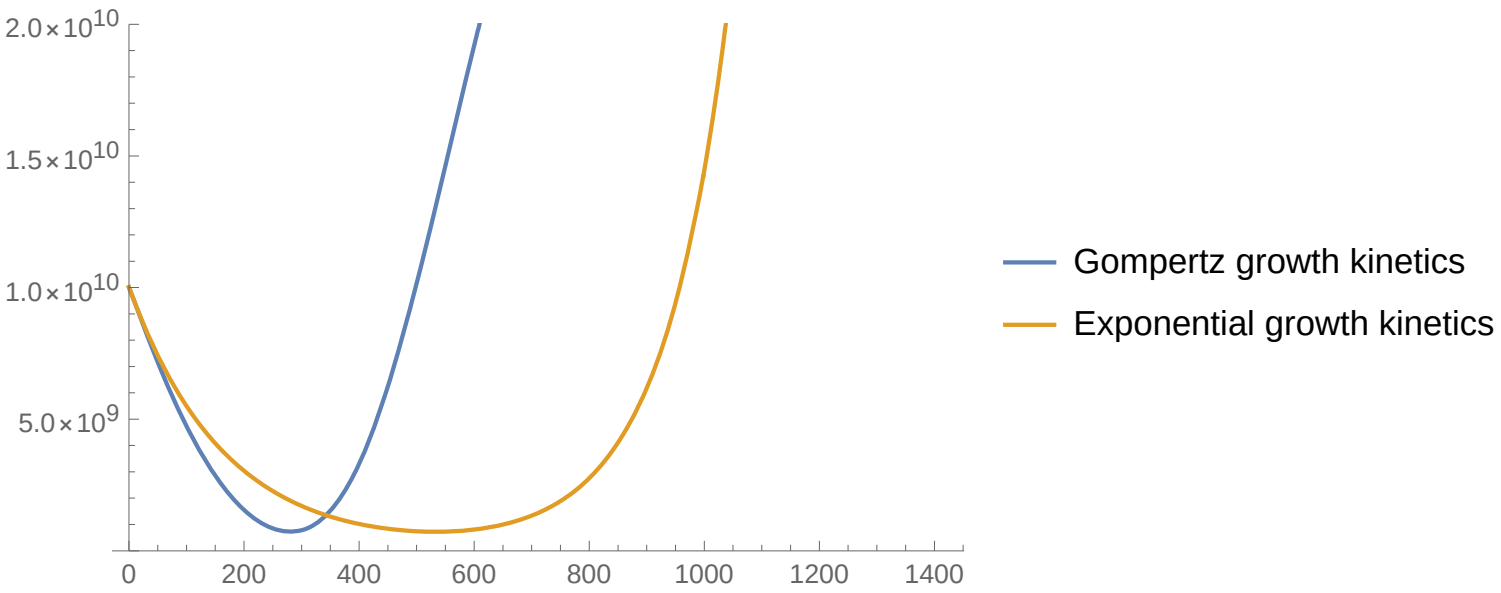
```
RGR[p_] := Max[{0, 1 - 1.66 * Log[10, p / p0]}];

(*drug dose, previously defined *)
d = DrugDose;

(* duration of numerical simulation *)
SimulationDuration = 4; (*years*)

(* simulating an untreated tumor *)
mysimresultUntreated =
 NDSolve[{p'[t] == gr * p[t] - dr * p[t], p[0] == p0}, p, {t, 0, SimulationDuration * 365}][[1]];

(* simulating growth of tumor cells with baseline drug sensitivity *)
mysimresult = NDSolve[{
    pS'[t] == gr * RGR[pS[t] + pR1[t] + pR2[t] + pRR[t]] * pS[t] *
       DrugTreatmentOverTime[t, LogDrugInhibitionModel /.
         {LogDrug → Log[10, d], LogKI → Min[{Log[10, KI1internal], Log[10, KI2internal]}],
          NH → 1}] - dr * RGR[pS[t] + pR1[t] + pR2[t] + pRR[t]] * pS[t],
    pS[0] == p0,
    pR1'[t] == gr * RGR[pS[t] + pR1[t] + pR2[t] + pRR[t]] * pR1[t] *
       DrugTreatmentOverTime[t, LogDrugInhibitionModel /.
         {LogDrug → Log[10, d], LogKI → Min[{Log[10, KI1internal * rl], Log[10, KI2internal]}],
          NH → 1}] - dr * RGR[pS[t] + pR1[t] + pR2[t] + pRR[t]] * pR1[t],
    pR1[0] == p0 * rf1,
    pR2'[t] == gr * RGR[pS[t] + pR1[t] + pR2[t] + pRR[t]] * pR2[t] *
       DrugTreatmentOverTime[t, LogDrugInhibitionModel /.
         {LogDrug → Log[10, d], LogKI → Min[{Log[10, KI1internal], Log[10, KI2internal * rl]}],
          NH → 1}] - dr * RGR[pS[t] + pR1[t] + pR2[t] + pRR[t]] * pR2[t],
    pR2[0] == p0 * rf2,
    pRR'[t] == gr * RGR[pS[t] + pR1[t] + pR2[t] + pRR[t]] * pRR[t] *
       DrugTreatmentOverTime[t, LogDrugInhibitionModel /.
         {LogDrug → Log[10, d],
          LogKI → Min[{Log[10, KI1internal * rl], Log[10, KI2internal * rl]}], NH → 1}] -
      dr * RGR[pS[t] + pR1[t] + pR2[t] + pRR[t]] * pRR[t],
    pRR[0] == p0 * rfCross
   }, {pS, pR1, pR2, pRR}, {t, 0, SimulationDuration * 365}][[1]];

(* Summing up the total population size at each time; sum of populations S, R1,
R2, RR. Table has an entry at every 2 days. *)
PopulationOverTimeTable = Table[{t, (pS[t] + pR1[t] + pR2[t] + pRR[t]) /. mysimresult},
   {t, 0, (SimulationDuration - 0.1) * 365, 2}];

 (* If progression threshold is not reached within the duration of the simulation,
 return a number larger than the duration of simulation
  (this can be used to identify such events) *)
 If[PopulationOverTimeTable[[-1, 2]] < ProgressionThreshold,
  Return[(SimulationDuration + 1) * 365]];

 (* final output: the earliest time at which the total population size surpasses
    the 'progression threshold', taken in this file to be twice the initial population,
 though it could be varied *)
 Select[PopulationOverTimeTable, #[[2]] > ProgressionThreshold &][[1, 1]]
]

(* a matching function where relative growth rate does not vary over time,
i.e. simple exponential growth. This is essentially the same as the simulation used
 in the main text but here the internal structure of the function is identical to
 the Gompertz simulation except that the 'RGR' function (relative growth rate) does
```

```
 not depend on population size. *)
ProgressionTimeWithoutGompertz[KI1_, KI2_, growthrate_, ProgressionThreshold_] := Module[{},
  KI1internal = KI1;
  KI2internal = KI2;
  (*cell division rate *)
  gr = growthrate;
  (*spontaneous cell death rate*)
  celllossrate = CellLossRate; (* previously defined as 80% *)
  (* death rate = growth rate × cell loss factor *)
  dr = gr * celllossrate;
  (* rl = 'resistance level', being the fold-change in KI in drug resistant sub-populations *)
  rl = 10;

  (* pre-existing resistance frequencies *)
  (* resistance to drug 1 *) rf1 = 500 * 10^-6;
  (* resistance to drug 2 *) rf2 = 500 * 10^-6;
  (* resistance to both drugs *) rfCross = 5 * 10^-6;

  (* initial population size *)
p0 = 10^10;

(* relative growth rate = RGR ; here is where we implement Gompertzian kinetics *)
RGR[p_] := 1;

(*drug dose, previously defined *)
d = DrugDose;

(* duration of numerical simulation *)
SimulationDuration = 4; (*years*)

(* simulating an untreated tumor *)
mysimresultUntreated =
 NDSolve[{p'[t] == gr * p[t] - dr * p[t], p[0] == p0}, p, {t, 0, SimulationDuration * 365}][[1]];

(* simulating growth of tumor cells with baseline drug sensitivity *)
mysimresult = NDSolve[{
    pS'[t] == gr * RGR[pS[t] + pR1[t] + pR2[t] + pRR[t]] * pS[t] *
       DrugTreatmentOverTime[t, LogDrugInhibitionModel /.
          {LogDrug → Log[10, d], LogKI → Min[{Log[10, KI1internal], Log[10, KI2internal]}],
           NH → 1}] - dr * RGR[pS[t] + pR1[t] + pR2[t] + pRR[t]] * pS[t],
   pS[0] == p0,
   pR1'[t] == gr * RGR[pS[t] + pR1[t] + pR2[t] + pRR[t]] * pR1[t] *
      DrugTreatmentOverTime[t, LogDrugInhibitionModel /.
         {LogDrug → Log[10, d], LogKI → Min[{Log[10, KI1internal * rl], Log[10, KI2internal]}],
          NH → 1}] - dr * RGR[pS[t] + pR1[t] + pR2[t] + pRR[t]] * pR1[t],
   pR1[0] == p0 * rf1,
   pR2'[t] == gr * RGR[pS[t] + pR1[t] + pR2[t] + pRR[t]] * pR2[t] *
      DrugTreatmentOverTime[t, LogDrugInhibitionModel /.
         {LogDrug → Log[10, d], LogKI → Min[{Log[10, KI1internal], Log[10, KI2internal * rl]}],
          NH → 1}] - dr * RGR[pS[t] + pR1[t] + pR2[t] + pRR[t]] * pR2[t],
   pR2[0] == p0 * rf2,
   pRR'[t] == gr * RGR[pS[t] + pR1[t] + pR2[t] + pRR[t]] * pRR[t] *
      DrugTreatmentOverTime[t, LogDrugInhibitionModel /.
         {LogDrug → Log[10, d],
          LogKI → Min[{Log[10, KI1internal * rl], Log[10, KI2internal * rl]}], NH → 1}] -
     dr * RGR[pS[t] + pR1[t] + pR2[t] + pRR[t]] * pRR[t],
   pRR[0] == p0 * rfCross
  }, {pS, pR1, pR2, pRR}, {t, 0, SimulationDuration * 365}][[1]];
```

```
 (* Summing up the total population size at each time; sum of populations S, R1,
 R2, RR. Table has an entry at every 2 days. *)
 PopulationOverTimeTable = Table[{t, (pS[t] + pR1[t] + pR2[t] + pRR[t]) /. mysimresult},
   {t, 0, (SimulationDuration - 0.1) * 365, 2}];

 (* If progression threshold is not reached within the duration of the simulation,
 return a number larger than the duration of simulation
  (this can be used to identify such events) *)
 If[PopulationOverTimeTable[[-1, 2]] < ProgressionThreshold,
  Return[(SimulationDuration + 1) * 365]];

 (* final output: the earliest time at which the total population size surpasses
    the 'progression threshold', taken in this file to be twice the initial population,
 though it could be varied *)
 Select[PopulationOverTimeTable, #[[2]] > ProgressionThreshold &][[1, 1]]
]
```

The faster kinetics that occur at small population size ('population rebound' time after effective treatment) can be seen in a side-by-side comparison of simulations conducted with identical input parameters (initial growth rate = 0.07, and drug sensitivities KI_1=2 and KI_2=0.5)

```
ProgressionTimeGompertz[2, 0.5, 0.07, 2 * 10^10];
OneTumorTimeCourseWithGompertz = PopulationOverTimeTable;
ProgressionTimeWithoutGompertz[2, 0.5, 0.07, 2 * 10^10];
OneTumorTimeCourseWithoutGompertz = PopulationOverTimeTable;

ListPlot[{OneTumorTimeCourseWithGompertz, OneTumorTimeCourseWithoutGompertz},
 PlotRange → {0, 2 * 10^10}, Joined → True,
 PlotLegends → {"Gompertz growth kinetics", "Exponential growth kinetics"}]
```

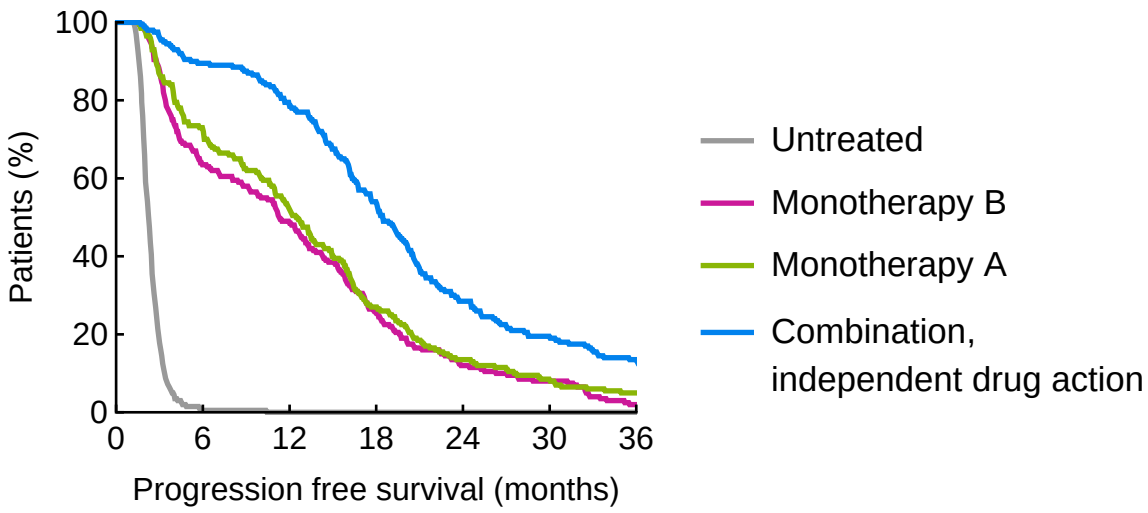



```
SimulatePopulationGompertz[] := Module[{KI1table, KI2table},

  KI1table =
   Table[RandomVariate[NormalDistribution[MedianLogDrugSensitivity,
      StandardDeviationInDrugSensitivity]], {1000}];
  KI2table =
   Table[RandomVariate[NormalDistribution[MedianLogDrugSensitivity,
      StandardDeviationInDrugSensitivity]], {1000}];

  logKI1 = 10^KI1table;
  logKI2 = 10^KI2table;

  NumberOfTumorsSimulated = 200;
```

```
ProgressionThreshold = 2 * 10^10;

(* simulating progression free survival time for no treatment. No treatment is
 implemented by setting KI1 and KI2 to equal 1000;
at this magnitude neither drug has any inhibitory effect on growth *)
UntreatedProgressionTimes =
 Table[ProgressionTimeGompertz[1000, 1000, DistributionOfGrowthRates[[i]], ProgressionThreshold],
  {i, 1, NumberOfTumorsSimulated}];
(* simulating progression free survival time for treatment by monotherapy #1. The
 absence of monotherapy #2 is implemented by setting KI2 to equal 1000;
at this magnitude drug 2 has no inhibitory effect on growth *)
Monotherapy1ProgressionTimes =
 Table[ProgressionTimeGompertz[logKI1[[i]], 1000, DistributionOfGrowthRates[[i]],
   ProgressionThreshold], {i, 1, NumberOfTumorsSimulated}];
(* simulating progression free survival time for treatment by monotherapy #2. The
 absence of monotherapy #1 is implemented by setting KI1 to equal 1000;
at this magnitude drug 2 has no inhibitory effect on growth *)
Monotherapy2ProgressionTimes =
 Table[ProgressionTimeGompertz[1000, logKI2[[i]], DistributionOfGrowthRates[[i]],
   ProgressionThreshold], {i, 1, NumberOfTumorsSimulated}];
(* simulating progression free survival time for treatment by a combination of
 monotherapy #1 and #2 *)
CombinationProgressionTimes =
 Table[ProgressionTimeGompertz[logKI1[[i]], logKI2[[i]], DistributionOfGrowthRates[[i]],
   ProgressionThreshold], {i, 1, NumberOfTumorsSimulated}];

(* Plotting survival functions *)
SurvivalPlot = ListPlot[{
   Table[{x, SurvivalFunction[EmpiricalDistribution[UntreatedProgressionTimes]][x]},
    {x, 0, 3.5 * 365, 2}],
   Table[{x, SurvivalFunction[EmpiricalDistribution[Monotherapy2ProgressionTimes]][x]},
    {x, 0, 3.5 * 365, 2}],
   Table[{x, SurvivalFunction[EmpiricalDistribution[Monotherapy1ProgressionTimes]][x]},
    {x, 0, 3.5 * 365, 2}],
   Table[{x, SurvivalFunction[EmpiricalDistribution[CombinationProgressionTimes]][x]},
    {x, 0, 3.5 * 365, 2}]
  },
  Joined → True, FrameTicks → {Table[{i, i * 2 / 61, {0.015, 0}}, {i, 0, 2000, 3 / 2 * 122}],
    Table[{i, 100 * i, {0.015, 0}}, {i, 0, 1, 1 / 5}]}, PlotRange → {{0, 36 * 30.5}, {0, 1}},
  Frame → {{True, False}, {True, False}}, FrameStyle → Directive[Black, Thickness[Medium]],
   BaseStyle → {FontFamily → "Arial", FontSize → 12}, AspectRatio → 3 / 4,
   FrameLabel → {"Progression free survival (months)", "Patients (%)"},
   ImageSize → {{250}, {250}}, ImagePadding → {{45, 10}, {45, 10}},
   PlotStyle → {Directive[GrayLevel[0.6], AbsoluteThickness[2]],
     Directive[RGBColor[0.8, 0.1, 0.6], AbsoluteThickness[2]],
     Directive[ColorData[3, 4], AbsoluteThickness[2]],
     Directive[ColorData[3, 6], AbsoluteThickness[2]]},
   PlotLegends → {"Untreated", "Monotherapy B", "Monotherapy A",
     "Combination,\nindependent drug action"}]
]
```

The benefit of independent drug action persists regardless of the choice of exponential or Gompertz growth kinetics :

```
(* Note that while the result of the simulation is time to tumor doubling,
the simulation (internally) proceeds for years,
producing numerically very large values for population size;
in the Gompertz model this generates some numerical computation errors as relative
 growth rate asymptotically approaches zero,
and so some error messages are here turned off. The time to doubling is calculated
 from data before these numerical errors take effect, and is therefore reported accurately *)
Off[Max::nord]
Off[InterpolatingFunction::dmval]
Off[NDSolve::nlnum]

SimulatePopulationGompertz[]
Export[NotebookDirectory[] <> "Supplementary Figure S7B, Gompertz PFS.pdf", %, "PDF"];
```



Spider plots, for monotherapy

Here we use precisely the same selection of growth rate and drug sensitivity parameters in Figure 6D, to compare tumor's drug responses in this alternative model structure

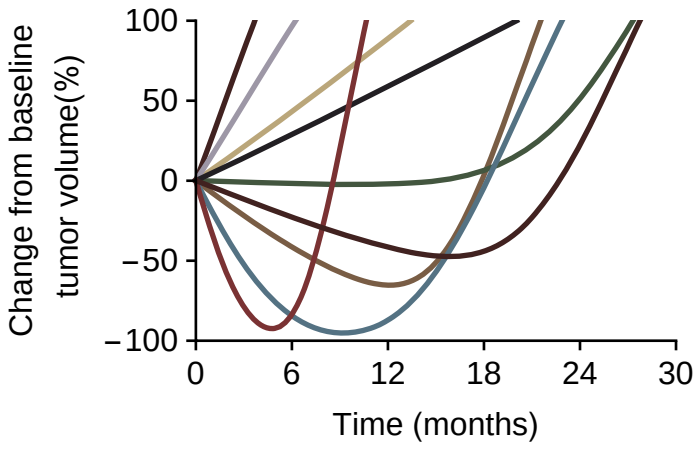
Because growth rates and drug sensitivities (KI) are randomly drawn from distributions, reproducible example trajectories can only be generated here by 'hard-coding' some sample values.

A small set of growth rates was generated by
Table[Max[{1/100,RandomVariate[NormalDistribution[6.9/100,2.1/100]]}],{50}];

A small set of KI values was generated by
10^Table[RandomVariate[NormalDistribution[MedianLogDrugSensitivity,StandardDeviationInDrug Sensitivity]],{50}]

These parameters generate 50 different tumor kinetic trajectories. A few of these trajectories have been selected here, non-randomly, to illustrate the variety of different kinetic trajectories.

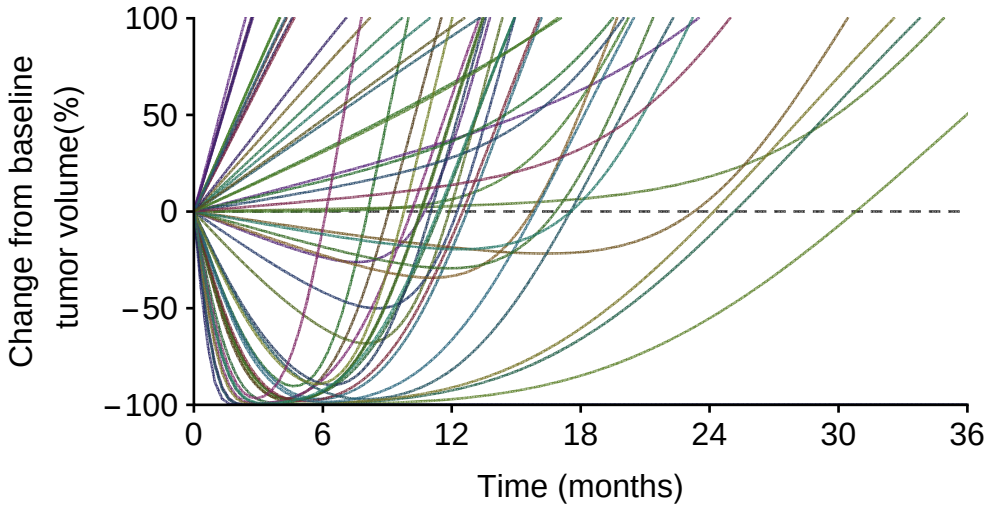
```
ASmallSetOfGrowthRates = {0.052`, 0.06`, 0.057`, 0.086`, 0.068`, 0.095`, 0.057`, 0.053`,
   0.045`, 0.033`, 0.083`, 0.084`, 0.061`, 0.094`, 0.092`, 0.077`, 0.088`, 0.1`, 0.098`,
   0.055`, 0.059`, 0.098`, 0.048`, 0.043`, 0.028`, 0.108`, 0.1`, 0.029`, 0.062`, 0.058`,
   0.045`, 0.063`, 0.088`, 0.079`, 0.038`, 0.057`, 0.121`, 0.075`, 0.054`, 0.101`, 0.063`,
   0.069`, 0.069`, 0.046`, 0.047`, 0.113`, 0.058`, 0.094`, 0.09`, 0.067`};

ASmallSetOfKIvalues = {5.41`, 3.84`, 0.62`, 0.95`, 1.83`, 3.46`, 0.79`, 1.06`, 0.38`,
   4.06`, 4.1`, 3.54`, 0.61`, 2.85`, 3.2`, 3.82`, 1.45`, 1.3`, 1.25`, 0.96`, 3.63`, 0.43`,
   0.68`, 2.53`, 3.96`, 1.14`, 1.69`, 0.32`, 1.38`, 0.4`, 0.96`, 1.68`, 0.63`, 2.1`,
   0.34`, 1.79`, 0.15`, 15.78`, 0.2`, 0.12`, 0.42`, 1.90`, 26.7`, 0.24`, 0.89`, 1.25`,
   4.62`, 0.93`, 0.8`, 2.62`};

Do[
 ProgressionTimeGompertz[ASmallSetOfKIvalues[[SimulatedTumorNumber]], 1000,
  ASmallSetOfGrowthRates[[SimulatedTumorNumber]], ProgressionThreshold];
 TotalPopulationOverTimeGompertz[SimulatedTumorNumber] =
  Table[{t, ((pS[t] + pR1[t] + pR2[t] + pRR[t]) /. mysimresult) / 10^10 - 1},
   {t, 0, (SimulationDuration - 0.5) * 365, 10}];
 SPopulationOverTimeGompertz[SimulatedTumorNumber] =
  Table[{t, (pS[t] /. mysimresult) / 10^10 - 1}, {t, 0, (SimulationDuration - 0.5) * 365, 10}];
 R1PopulationOverTimeGompertz[SimulatedTumorNumber] =
  Table[{t, (pR1[t] /. mysimresult) / 10^10 - 1}, {t, 0, (SimulationDuration - 0.5) * 365, 10}];
 R2PopulationOverTimeGompertz[SimulatedTumorNumber] =
  Table[{t, (pR2[t] /. mysimresult) / 10^10 - 1}, {t, 0, (SimulationDuration - 0.5) * 365, 10}];
 RRPopulationOverTimeGompertz[SimulatedTumorNumber] =
  Table[{t, (pRR[t] /. mysimresult) / 10^10 - 1}, {t, 0, (SimulationDuration - 0.5) * 365, 10}];

 , {SimulatedTumorNumber, 1, 50}]

(* a subset was selected (non-randomly) to illustrate the diversity of different
 treatment outcomes *)
ListPlot[Table[TotalPopulationOverTimeGompertz[stn], {stn, {1, 3, 4, 7, 9, 10, 20, 22, 23}}],
 PlotRange → {{0, 30 * 30.5}, {-1, 1}}, Joined → True,
 PlotStyle →
  Table[Directive[Blend[{ColorData[16, i], ColorConvert[ColorData[16, i], "Grayscale"]}, 0.7],
    Opacity[1], AbsoluteThickness[2]], {i, {1, 2, 3, 4, 6, 7, 8, 9, 10}}],
 Frame → {{True, False}, {True, False}}, Axes → {False, False}, Ticks → None,
 PlotRangePadding → {{0, 0}, {0.0, 0}}, FrameStyle → Directive[Black, Thickness[Medium]],
 AxesStyle -> Directive[Black, Thickness[Medium]],
 BaseStyle → {FontFamily → "Arial", FontSize → 12},
 FrameTicks → {{Table[{i, 100 * i, {0, 0.02}}, {i, -1, 1, 1 / 2}], None},
   {Table[{i, i * 2 / 61, {0, 0.02}}, {i, 0, 2000, 6 / 4 * 122}], None}}, AspectRatio → 2 / 3,
 ImageSize → {{1000}, {200}}, ImagePadding → {{100, 10}, {60, 20}},
 FrameLabel → {"Time (months)", "Change from baseline\n tumor volume(%)"},
 Prolog → {Dashing[{0.015, 0.01}], Opacity[0.7], GrayLevel[0.], Thickness[Medium](*,
   Line[{{0,0},{2*366,0}}]*)}]

Export[NotebookDirectory[] <> "Supplementary Figure S7B, Gompertz kinetics.pdf", %, "PDF"];
```



A larger batch produced by randomly drawn parameters

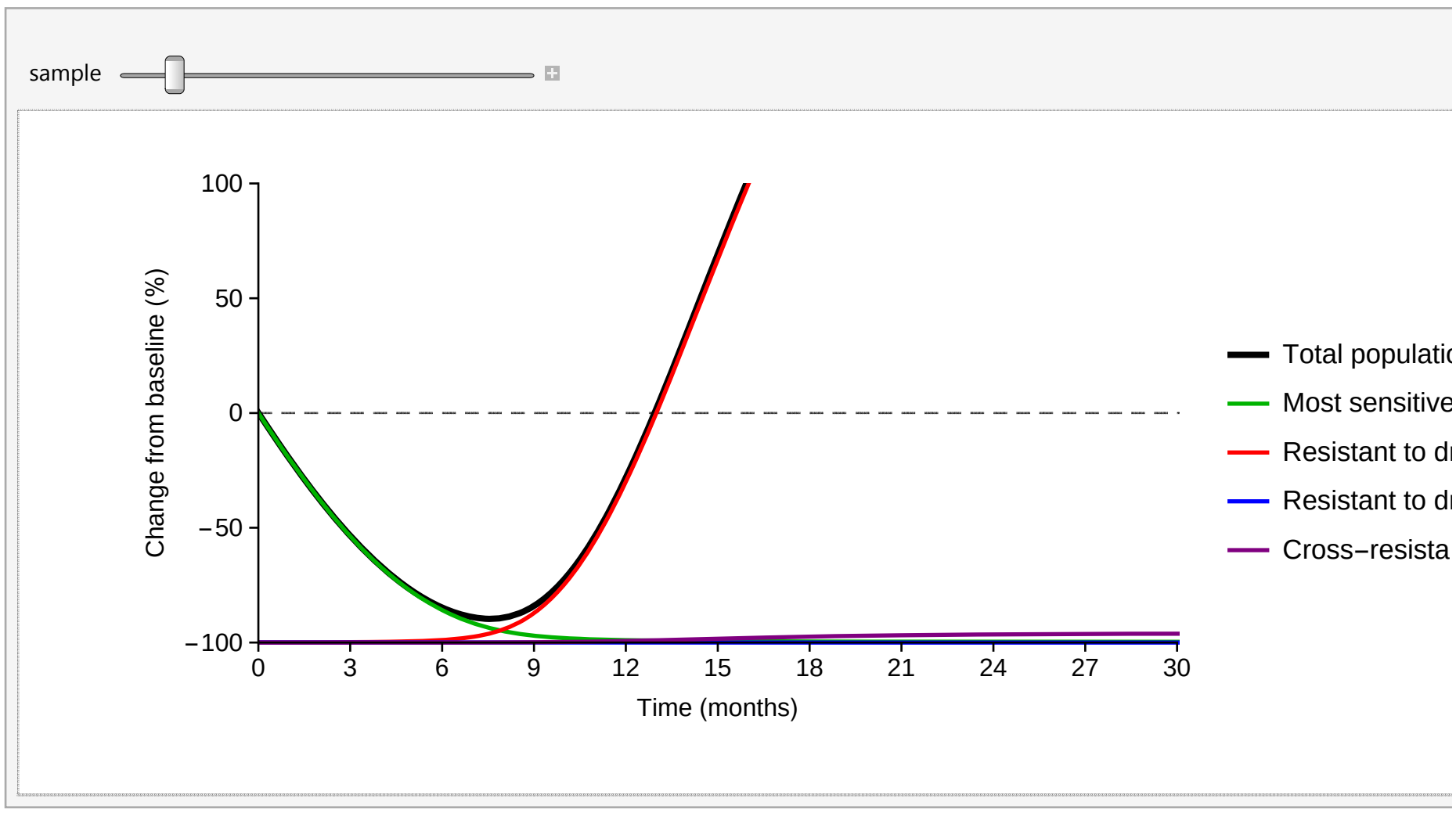
```
Do[
 ProgressionTimeGompertz[logKI1[[SimulatedTumorNumber]], 1000,
  DistributionOfGrowthRates[[SimulatedTumorNumber]], ProgressionThreshold];
 TotalPopulationOverTimeGompertz[SimulatedTumorNumber] =
  Table[{t, ((pS[t] + pR1[t] + pR2[t] + pRR[t]) /. mysimresult) / 10^10 - 1},
   {t, 0, (SimulationDuration - 0.5) * 365, 10}];
 SPopulationOverTimeGompertz[SimulatedTumorNumber] =
  Table[{t, (pS[t] /. mysimresult) / 10^10 - 1}, {t, 0, (SimulationDuration - 0.5) * 365, 10}];
 R1PopulationOverTimeGompertz[SimulatedTumorNumber] =
  Table[{t, (pR1[t] /. mysimresult) / 10^10 - 1}, {t, 0, (SimulationDuration - 0.5) * 365, 10}];
 R2PopulationOverTimeGompertz[SimulatedTumorNumber] =
  Table[{t, (pR2[t] /. mysimresult) / 10^10 - 1}, {t, 0, (SimulationDuration - 0.5) * 365, 10}];
 RRPopulationOverTimeGompertz[SimulatedTumorNumber] =
  Table[{t, (pRR[t] /. mysimresult) / 10^10 - 1}, {t, 0, (SimulationDuration - 0.5) * 365, 10}];

, {SimulatedTumorNumber, 1, 200}]
```

```
ListPlot[Table[TotalPopulationOverTimeGompertz[SimulatedTumorNumber],
  {SimulatedTumorNumber, 50, 100}], PlotRange → {{0, 3 * 366}, {-1, 1}}, Joined → True,
 PlotStyle → Table[Directive[Hue[RandomReal[{0, 1}], 0.7, RandomReal[{0.3, 0.5}]],
    Opacity[0.7], Thickness[Medium]], {200}], Frame → {{True, False}, {True, False}},
 Axes → {False, False}, Ticks → None, PlotRangePadding → None,
 FrameStyle → Directive[Black, Thickness[Medium]],
 AxesStyle -> Directive[Black, Thickness[Medium]],
 BaseStyle → {FontFamily → "Arial", FontSize → 12},
 FrameTicks → {{Table[{i, 100 * i, {0, 0.01}}, {i, -1, 1, 1 / 2}], None},
   {Table[{i, i * 2 / 61, {0, 0.01}}, {i, 0, 2000, 3 / 2 * 122}], None}}, AspectRatio → 1 / 2,
 ImageSize → {{400}, {500}}, ImagePadding → {{100, 10}, {60, 20}},
 FrameLabel → {"Time (months)", "Change from baseline\n tumor volume(%)"},
 Prolog → {Dashing[{0.015, 0.01}], Opacity[0.7], GrayLevel[0.], Thickness[Medium],
   Line[{{0, 0}, {3 * 366, 0}}]}]
```



This interface allows the user to scroll through different individual tumors and inspect the growth (or inhibition) of its sub - populations over time.

```
Manipulate[
 ListPlot[{TotalPopulationOverTimeGompertz[sample], SPopulationOverTimeGompertz[sample],
   R1PopulationOverTimeGompertz[sample], R2PopulationOverTimeGompertz[sample],
   RRPopulationOverTimeGompertz[sample]}, PlotRange → {{0, 30 * 30.5}, {-1, 1}},
  Joined → True,
  PlotStyle → Join[{Directive[Black, Opacity[1], AbsoluteThickness[3]]},
    Table[Directive[col, Opacity[1], AbsoluteThickness[2]],
     {col, {Darker[Green, 0.3], Red, Blue, Purple}}]], Frame → {{True, False}, {True, False}},
  Axes → {False, False}, Ticks → None, PlotRangePadding → {{0, 0}, {0.0, 0}},
  FrameStyle → Directive[Black, Thickness[Medium]],
  AxesStyle -> Directive[Black, Thickness[Medium]],
  BaseStyle → {FontFamily → "Arial", FontSize → 12},
  FrameTicks → {{Table[{i, 100 * i, {0, 0.01}}, {i, -1, 1, 1 / 2}], None},
    {Table[{i, i * 2 / 61, {0, 0.01}}, {i, 0, 2000, 3 / 4 * 122}], None}}, AspectRatio → 1 / 2,
  ImageSize → {{1000}, {300}}, ImagePadding → {{100, 10}, {60, 20}},
  FrameLabel → {"Time (months)", "Change from baseline (%)"},
  Prolog → {Dashing[{0.015, 0.01}], Opacity[0.7], GrayLevel[0.], Thickness[Medium],
    Line[{{0, 0}, {3 * 366, 0}}]},
  PlotLegends → {"Total population", "Most sensitive (S)", "Resistant to drug 1 (R1)",
    "Resistant to drug 2 (R2)", "Cross-resistant (RR)"}]
, {sample, 1, 200, 1}]
```



A model of treatment with cytotoxic chemotherapy in cycles

Here we alter two features of the model presented in Figure 6:

(1) Drug response: treatment does not only arrest tumor cell proliferation, but actively induces cell death

(2) Drug administration: treatment is not present continuously, but is administered once a month (and in this model, exerts a cell killing effect for 3 days; this parameter can be varied)

(1) Drug response.

This model of cytotoxic drug response takes the following simple form:

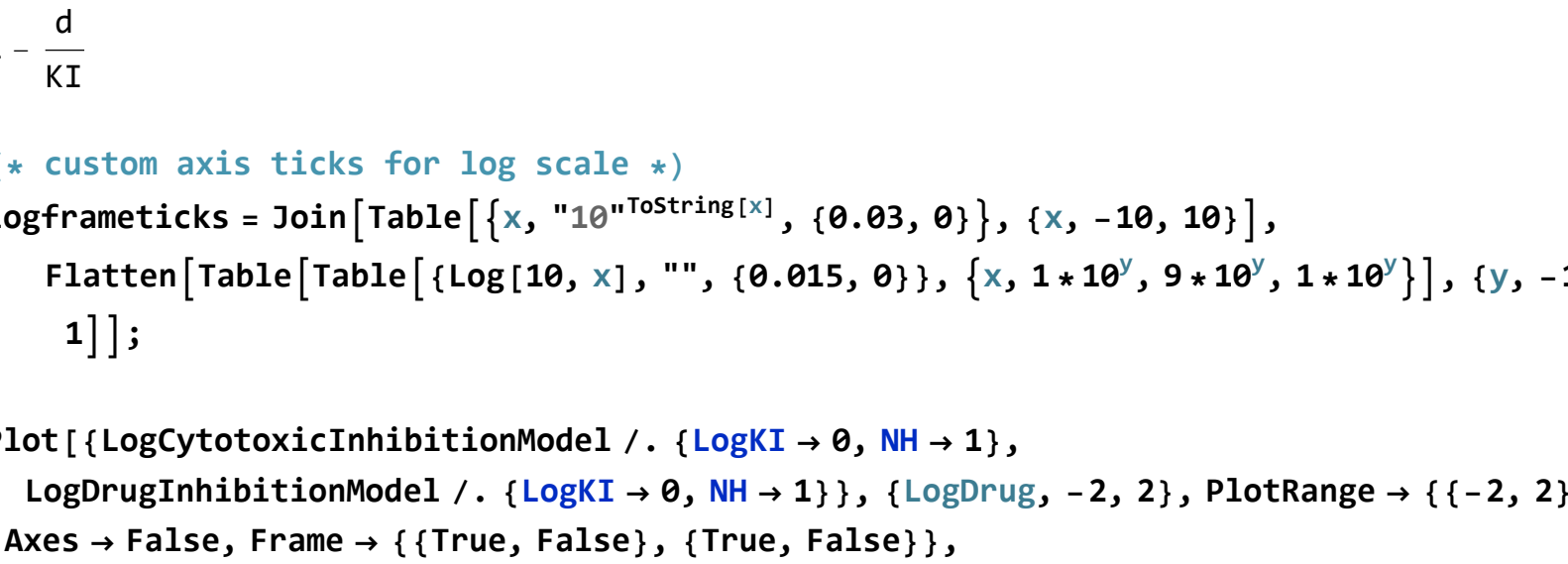
$$\text{Relative tumor growth rate} = 1 - \frac{[\text{drug concentration}]}{K_I}$$

Note that in this model of cytotoxic drug response, 'relative tumor growth' reaches negative numbers at high drug concentration. These negative numbers are interpreted by recalling that such a scale refers to the relative change in tumor cell population in each cell division time; thus the cell population decreases rather than increases in each cell division time, even before accounting for the cell loss factor (which is a separate source of population decrease)
For example,
1 = uninhibited growth; population doubles in each cell division time (before accounting for cell loss)
0 = growth arrest (no cell division; before accounting for cell loss)
-1 = population halves in each cell division time (before accounting for cell loss). Equivalent to growth arrest plus 50% cell killing in each cell division time.
-2 = population quartered in each cell division time. Equivalent to growth arrest plus 75% cell killing in each cell division time.
etc.

```
LogCytotoxicInhibitionModel = 1 - (10^(LogDrug-LogKI))^NH;

Clear[d]
Simplify[(LogCytotoxicInhibitionModel) /. {NH → 1, LogKI → Log[10, KI], LogDrug → Log[10, d]}]
```

$$1 - \frac{d}{KI}$$

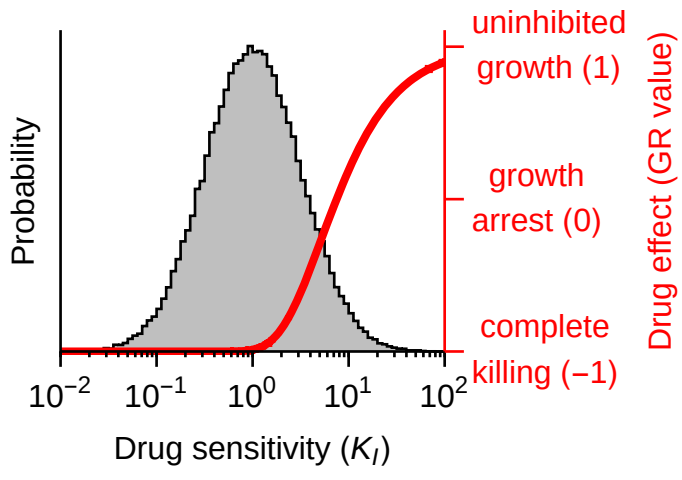
```
(* custom axis ticks for log scale *)
logframeticks = Join[Table[{x, "10"^ToString[x], {0.03, 0}}, {x, -10, 10}],
   Flatten[Table[Table[{Log[10, x], "", {0.015, 0}}, {x, 1 * 10^y, 9 * 10^y, 1 * 10^y}], {y, -10, 10, 1}],
    1]];

Plot[{LogCytotoxicInhibitionModel /. {LogKI → 0, NH → 1},
  LogDrugInhibitionModel /. {LogKI → 0, NH → 1}}, {LogDrug, -2, 2}, PlotRange → {{-2, 2}, {-2, 1}},
 Axes → False, Frame → {{True, False}, {True, False}},
 FrameStyle → Directive[Black, Thickness[Medium]],
 BaseStyle → {FontFamily → "Arial", FontSize → 12}, AspectRatio → 3 / 4,
 FrameLabel → {"Drug concentration (as multiples of K_I)",
   Rotate[
    "Relative tumor\ngrowth rate\n(negative indicates\ncell killing;\ne.g. -1
      implies\n50% cell killing\neach cell\ndivision time)", -π / 2]},
 FrameTicks → {logframeticks, Table[{i, NumberForm[i, {2, 1}], {0.02, 0}}, {i, -3, 1, 0.5}]},
 Epilog → {GrayLevel[0.5], Thickness[Medium], Line[{{-2, 0}, {2, 0}}]},
 PlotLegends → {"Model of cytotoxic drug response", "Model of cytostatic drug response"}]
```



In comparison to the model in the main text (Figure 6), where drug administration was continuous, here a higher drug concentration is required to have a similar effect because of its transient effect (acting for 3 days out of every 30)

```
(* When drug is present only 3 days out of every 30,
a significant response is only observed with a dose that is higher (relative to KI)
 than was used in the model of continuously administered therapy (as in Figure 6) *)
DrugDose = 1.5;
```

```
logframeticksDownward = Join[Table[{x, "10"^ToString[x], {0, 0.04}}, {x, -10, 10}],
   Flatten[Table[Table[{Log[10, x], "", {0, 0.015}}, {x, 1 * 10^y, 9 * 10^y, 1 * 10^y}], {y, -10, 10, 1}],
    1]];

LogNormallyDistributedKIvalues =
  Table[RandomVariate[NormalDistribution[MedianLogDrugSensitivity,
     StandardDeviationInDrugSensitivity]], {100000}];

CustomHistogramPlot[data_, bins_] :=
 Module[
  {countBorder = Partition[Riffle[Riffle[#1, #1[[2 ;;]]], Riffle[#2, #2]], 2] & @@
     HistogramList[data, bins, "Count"]},
  ListLinePlot[countBorder, Filling → Axis, FillingStyle → Directive[GrayLevel[0.75], Opacity[1]],
   PlotStyle -> Directive[Opacity[1], Black, AbsoluteThickness[1]],
   Frame → {{True, True}, {True, False}}, Axes → {False, False},
   FrameTicks →
    {{None, (Table[{i * 2000 + 2000, Style[ToString[i], Red], {0, 0.05}}, {i, -1, 1, 1}] /.
        {"0" → "growth\narrest (0)", "1" → Style["uninhibited\ngrowth (1)", LineSpacing → {4, 0}],
         "-1" → "complete\nkilling (-1)"})}, {logframeticksDownward, None}},
   PlotRangePadding → None,
   FrameStyle → {{Directive[GrayLevel[0.0], Thickness[Medium]],
      Directive[Red, Thickness[Medium]]},
     {Directive[Black, Thickness[Medium]], Directive[Black, Thickness[Medium]]}},
   BaseStyle → {FontFamily → "Arial", FontSize → 12},
   FrameLabel → {{Style["Probability", GrayLevel[0.]], Style["Drug effect (GR value)", Red]},
     {"Drug sensitivity (K_I)",}}, AspectRatio → 5 / 6, ImageSize → {{1000}, {200}},
   ImagePadding → {{50, 110}, {60, 20}}, PlotRange → {{Log[10, 1 / 100], Log[10, 100]}, {0, 4200}}]]

SensitivityDistributionAndDrugResponsePlot = Show[
  CustomHistogramPlot[LogNormallyDistributedKIvalues, {-2, 2, 0.05}]
  ,
  Plot[
   (2^(((LogCytotoxicInhibitionModel /. {LogDrug → Log[10, DrugDose], NH → 1}) - CellLossRate) /
         (1 - CellLossRate)) - 1) * 2000 + 2000, {LogKI, Log[10, 1 / 100], Log[10, 100]},
   Frame → {{True, False}, {True, False}}, Axes → {True, False},
   AxesStyle → Directive[Black, Thickness[Medium]],
   FrameTicks → {{None, Table[{i, Style[ToString[i], Black], {0, 0.02}}, {i, -1, 1, 1}]},
     {logframeticks, None}}, PlotRangePadding → None, PlotRange → {{-2, 2}, {0, All}},
   FrameStyle → Directive[Black, Thickness[Medium]],
   BaseStyle → {FontFamily → "Arial", FontSize → 12},
   FrameLabel → {{"Drug sensitivity (K_I)",}, {"Probability", "GR"}}, AspectRatio → 3 / 4,
   ImageSize → {{250}, {250}}, ImagePadding → {{50, 10}, {60, 10}},
   PlotStyle → Directive[Red, AbsoluteThickness[3]]]
]
```



(2) Drug administration

Note that drug dose response extends 'tumor growth growth' to negative numbers; which refer to the relative change in tumor cell population in each cell division time. For example,

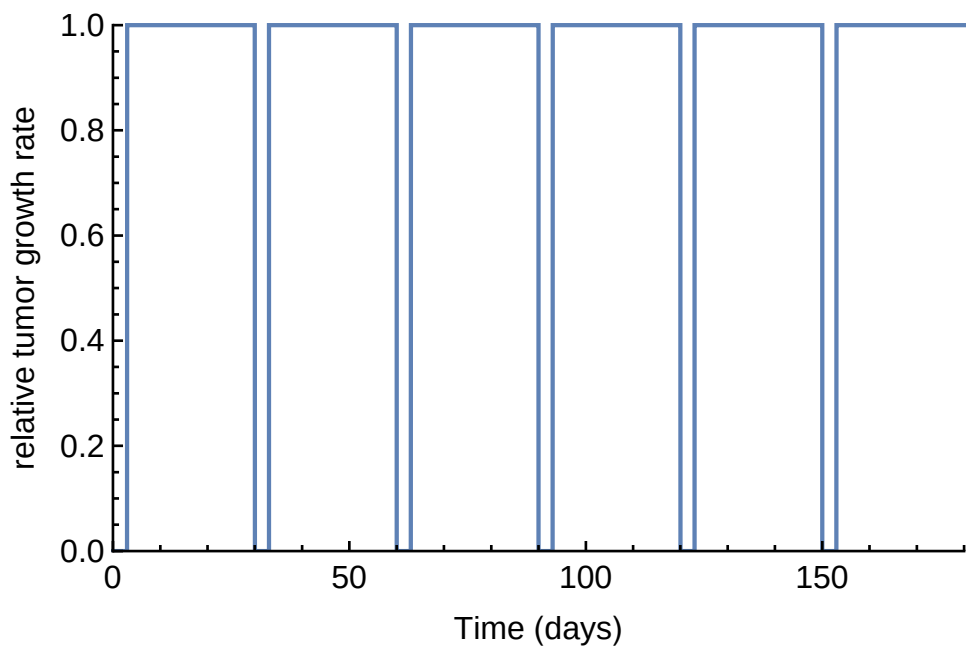
1 = uninhibited growth; population doubles in each cell division time (before accounting for cell loss factor)

0 = growth arrest (no cell division; before accounting for cell loss)

-1 = population halves in each cell division time (before accounting for cell loss). Equivalent to growth arrest plus 50% cell killing in each cell division time.

-2 = population quartered in each cell division time. Equivalent to growth arrest plus 75% cell killing in each cell division time.

```
(* here the drug's effect is present for 3 days out of every 30*)
DurationOfDrugEffectPerCycle = 3;
CycleLength = 30;
NumberOfCycles = 36; (*'36' cycles indicates that treatment cycles continue for
 3 years (essentially indefinitely given the timescale being simulated);
this parameter could be adjusted to describe treatment with a smaller fixed number of cycles;
the benefit of independent drug action persists in such models *)
DrugTreatmentOverTime[t_, RelativeGrowthDuringTreatment_] :=
 If[And[Mod[t, CycleLength] <= DurationOfDrugEffectPerCycle,
   t < (NumberOfCycles + 0.5) * CycleLength], RelativeGrowthDuringTreatment, 1]

(* a simple illustration of a drug's presence and effect over time: *)
Plot[DrugTreatmentOverTime[t, 0], {t, 0, 6 * 30}, PlotPoints → 200,
 Frame → {{True, False}, {True, False}}, Exclusions → None, PlotRange → All,
 FrameStyle → Directive[Black, Thickness[Medium]],
 BaseStyle → {FontFamily → "Arial", FontSize → 12}, PlotRangePadding → None,
 FrameLabel → {"Time (days)", "relative tumor growth rate"}]
```




```
ProgressionTimeCytotoxic[KI1_, KI2_, growthrate_, ProgressionThreshold_] := Module[{},
  KI1internal = KI1;
  KI2internal = KI2;
  (*cell division rate *)
  gr = growthrate;
  (*spontaneous cell death rate*)
  celllossrate = CellLossRate; (* previously defined as 80% *)
  (* death rate = growth rate × cell loss factor *)
  dr = gr * celllossrate;
  (* rl = 'resistance level', being the fold-change in KI in drug resistant sub-populations *)
  rl = 10;

  (* pre-existing resistance frequencies *)
  (* resistance to drug 1 *) rf1 = 500 * 10^-6;
```

```
(* resistance to drug 2 *) rf2 = 500 * 10^-6;
(* resistance to both drugs *) rfCross = 5 * 10^-6;

(* initial population size *)
p0 = 10^10;
(*drug dose, previously defined *)
d = DrugDose;

(* duration of numerical simulation *)
SimulationDuration = 4; (*years*)

(* simulating an untreated tumor *)
mysimresultUntreated =
 NDSolve[{p'[t] == gr * p[t] - dr * p[t], p[0] == p0}, p, {t, 0, SimulationDuration * 365}][[1]];

(* simulating growth of tumor cells with baseline drug sensitivity *)
mysimresultS =
 NDSolve[
   {p'[t] ==
     gr * p[t] * DrugTreatmentOverTime[t, LogCytotoxicInhibitionModel /.
         {LogDrug → Log[10, d], LogKI → Min[{Log[10, KI1internal], Log[10, KI2internal]}],
          NH → 1}] - dr * p[t], p[0] == p0}, p, {t, 0, SimulationDuration * 365}][[1]];
(* simulating growth of subpopulation with resistance to drug 1 *)
mysimresultR1 =
 NDSolve[
   {p'[t] ==
     gr * p[t] * DrugTreatmentOverTime[t, LogCytotoxicInhibitionModel /.
         {LogDrug → Log[10, d], LogKI → Min[{Log[10, KI1internal * rl], Log[10, KI2internal]}],
          NH → 1}] - dr * p[t], p[0] == p0 * rf1}, p, {t, 0, SimulationDuration * 365}][[1]];
(* simulating growth of subpopulation with resistance to drug 2 *)
mysimresultR2 =
 NDSolve[
   {p'[t] ==
     gr * p[t] * DrugTreatmentOverTime[t, LogCytotoxicInhibitionModel /.
         {LogDrug → Log[10, d], LogKI → Min[{Log[10, KI1internal], Log[10, KI2internal * rl]}],
          NH → 1}] - dr * p[t], p[0] == p0 * rf2}, p, {t, 0, SimulationDuration * 365}][[1]];
(* simulating growth of subpopulation with resistance to both drugs *)
mysimresultRR =
 NDSolve[
   {p'[t] ==
     gr * p[t] * DrugTreatmentOverTime[t, LogCytotoxicInhibitionModel /.
         {LogDrug → Log[10, d],
          LogKI → Min[{Log[10, KI1internal * rl], Log[10, KI2internal * rl]}], NH → 1}] - dr * p[t],
    p[0] == p0 * rfCross}, p, {t, 0, SimulationDuration * 365}][[1]];

(* Summing up the total population size at each time; sum of populations S, R1,
R2, RR. Table has an entry at every 2 days. *)
PopulationOverTimeTable =
 Table[{t, (p[t] /. mysimresultS) + (p[t] /. mysimresultR1) + (p[t] /. mysimresultR2) +
    (p[t] /. mysimresultRR)}, {t, 0, (SimulationDuration - 0.1) * 365, 2}];

(* If progression threshold is not reached within the duration of the simulation,
return a number larger than the duration of simulation
 (this can be used to identify such events) *)
If[PopulationOverTimeTable[[-1, 2]] < ProgressionThreshold,
 Return[(SimulationDuration + 1) * 365]];

(* final output: the earliest time at which the total population size surpasses
```

```
    the 'progression threshold', taken in this file to be twice the initial population,
  though it could be varied *)
  Select[PopulationOverTimeTable, #[[2]] > ProgressionThreshold &][[1, 1]]
 ]
```

‘SimulatePopulation’ executes the above simulation (ProgressionTime) for each individual in a population of 200.
It takes a single input parameter, being the correlation between KI for drug 1 and KI for drug 2

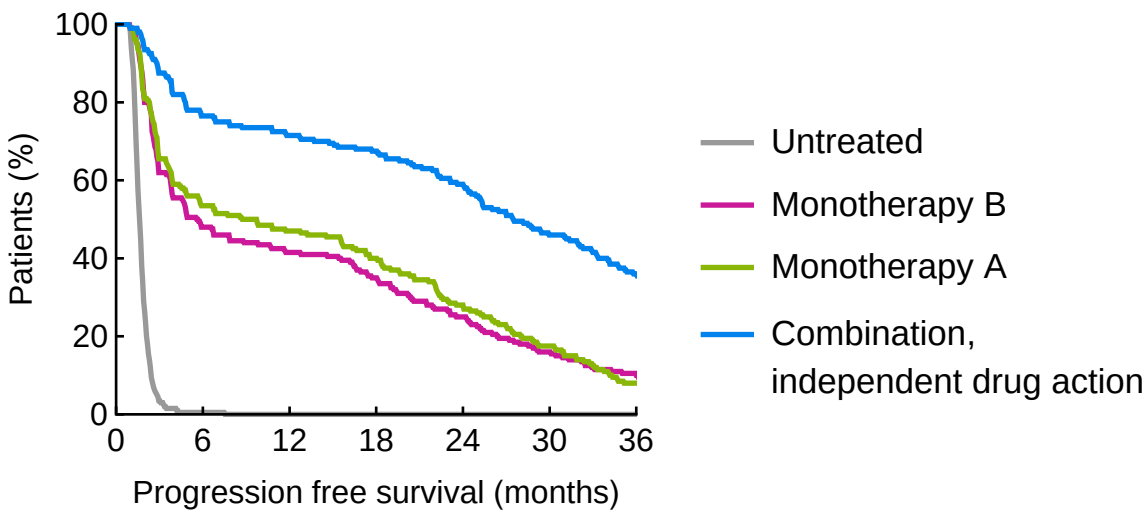
```
SimulatePopulationCytotoxic[] := Module[{KI1table, KI2table},

  KI1table =
   Table[RandomVariate[NormalDistribution[MedianLogDrugSensitivity,
      StandardDeviationInDrugSensitivity]], {1000}];
  KI2table =
   Table[RandomVariate[NormalDistribution[MedianLogDrugSensitivity,
      StandardDeviationInDrugSensitivity]], {1000}];

  logKI1 = 10^KI1table;
  logKI2 = 10^KI2table;

  NumberOfTumorsSimulated = 200;
  ProgressionThreshold = 2 * 10^10;

  (* simulating progression free survival time for no treatment. No treatment is
   implemented by setting KI1 and KI2 to equal 1000;
  at this magnitude neither drug has any inhibitory effect on growth *)
  UntreatedProgressionTimes =
   Parallelize[Table[ProgressionTimeCytotoxic[1000, 1000, DistributionOfGrowthRates[[i]],
      ProgressionThreshold], {i, 1, NumberOfTumorsSimulated}]];
  (* simulating progression free survival time for treatment by monotherapy #1. The
   absence of monotherapy #2 is implemented by setting KI2 to equal 1000;
  at this magnitude drug 2 has no inhibitory effect on growth *)
  Monotherapy1ProgressionTimes =
   Parallelize[Table[ProgressionTimeCytotoxic[logKI1[[i]], 1000, DistributionOfGrowthRates[[i]],
      ProgressionThreshold], {i, 1, NumberOfTumorsSimulated}]];
  (* simulating progression free survival time for treatment by monotherapy #2. The
   absence of monotherapy #1 is implemented by setting KI1 to equal 1000;
  at this magnitude drug 2 has no inhibitory effect on growth *)
  Monotherapy2ProgressionTimes =
   Parallelize[Table[ProgressionTimeCytotoxic[1000, logKI2[[i]], DistributionOfGrowthRates[[i]],
      ProgressionThreshold], {i, 1, NumberOfTumorsSimulated}]];
  (* simulating progression free survival time for treatment by a combination of
   monotherapy #1 and #2 *)
  CombinationProgressionTimes =
   Parallelize[Table[ProgressionTimeCytotoxic[logKI1[[i]], logKI2[[i]],
      DistributionOfGrowthRates[[i]], ProgressionThreshold], {i, 1, NumberOfTumorsSimulated}]];

  (* Plotting survival functions *)
  SurvivalPlot = ListPlot[{
     Table[{x, SurvivalFunction[EmpiricalDistribution[UntreatedProgressionTimes]][x]},
      {x, 0, 3.5 * 365, 2}],
     Table[{x, SurvivalFunction[EmpiricalDistribution[Monotherapy2ProgressionTimes]][x]},
      {x, 0, 3.5 * 365, 2}],
     Table[{x, SurvivalFunction[EmpiricalDistribution[Monotherapy1ProgressionTimes]][x]},
      {x, 0, 3.5 * 365, 2}],
     Table[{x, SurvivalFunction[EmpiricalDistribution[CombinationProgressionTimes]][x]},
      {x, 0, 3.5 * 365, 2}]
```

```
    },
    Joined → True, FrameTicks → {Table[{i, i * 2 / 61, {0.015, 0}}, {i, 0, 2000, 3 / 2 * 122}],
      Table[{i, 100 * i, {0.015, 0}}, {i, 0, 1, 1 / 5}]}, PlotRange → {{0, 36 * 30.5}, {0, 1}},
    Frame → {{True, False}, {True, False}}, FrameStyle → Directive[Black, Thickness[Medium]],
    BaseStyle → {FontFamily → "Arial", FontSize → 12}, AspectRatio → 3 / 4,
    FrameLabel → {"Progression free survival (months)", "Patients (%)"},
    ImageSize → {{250}, {250}}, ImagePadding → {{45, 10}, {45, 10}},
    PlotStyle → {Directive[GrayLevel[0.6], AbsoluteThickness[2]],
      Directive[RGBColor[0.8, 0.1, 0.6], AbsoluteThickness[2]],
      Directive[ColorData[3, 4], AbsoluteThickness[2]],
      Directive[ColorData[3, 6], AbsoluteThickness[2]]},
    PlotLegends → {"Untreated", "Monotherapy B", "Monotherapy A",
      "Combination,\nindependent drug action"}]
]
```

The benefit of independent drug action in a combination is present also in a model of cycles of cytotoxic therapy:

```
SimulatePopulationCytotoxic[]
```

```
Export[NotebookDirectory[] <> "Supplementary Figure S7B, Cytotoxic model PFS.pdf", %, "PDF"];
```



Spider plots, for monotherapy

Here we use precisely the same selection of growth rate and drug sensitivity parameters in Figure 6D, to compare tumor's drug responses in this alternative model structure

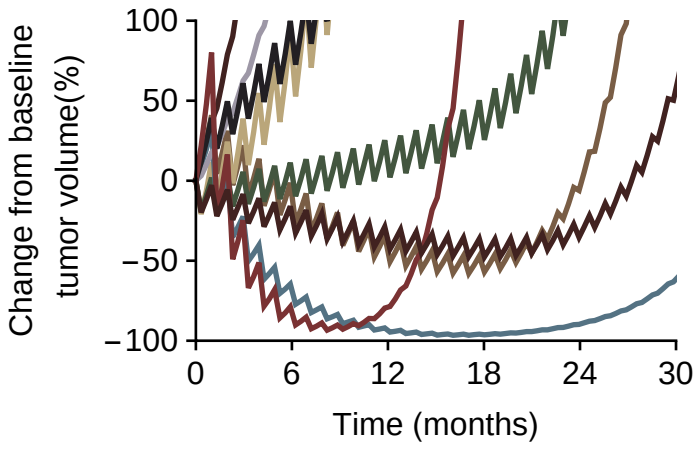
Because growth rates and drug sensitivities (KI) are randomly drawn from distributions, reproducible example trajectories can only be generated here by 'hard-coding' some sample values.
Note: in the simulation of cycles of cytotoxic therapy, numerical error is larger than in simulations of continuous therapy because of the discontinuity in drug administration (3 days in every 30) - this introduces minor variation between repeat executions of the simulation.

A small set of growth rates was generated by
Table[Max[{1/100,RandomVariate[NormalDistribution[6.9/100,2.1/100]]}],{50}];

A small set of KI values was generated by
10^Table[RandomVariate[NormalDistribution[MedianLogDrugSensitivity,StandardDeviationInDrugSensitivity]],{50}]

These parameters generate 50 different tumor kinetic trajectories. A few of these trajectories have been selected here, non-randomly, to illustrate the variety of different kinetic trajectories.

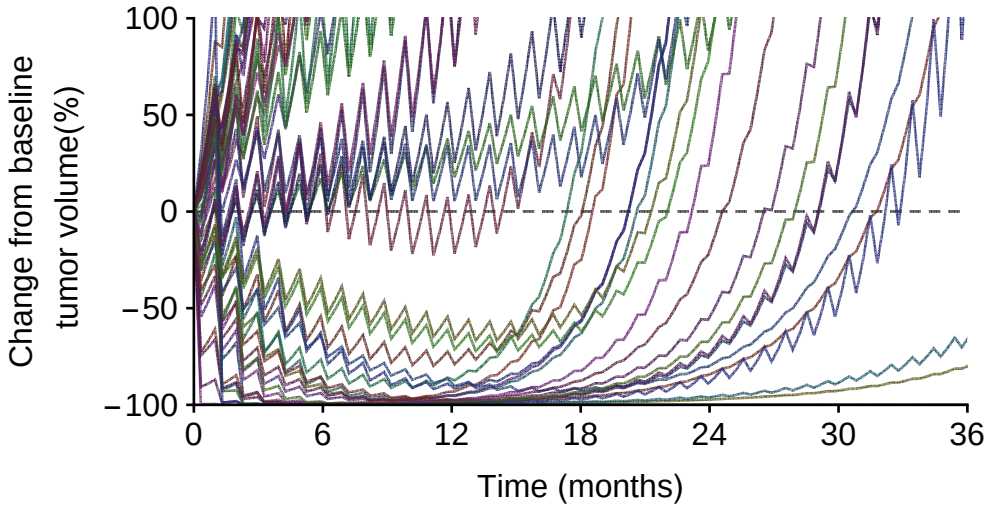
```
ASmallSetOfGrowthRates = {0.052`, 0.06`, 0.057`, 0.086`, 0.068`, 0.095`, 0.057`, 0.053`,
   0.045`, 0.033`, 0.083`, 0.084`, 0.061`, 0.094`, 0.092`, 0.077`, 0.088`, 0.1`, 0.098`,
   0.055`, 0.059`, 0.098`, 0.048`, 0.043`, 0.028`, 0.108`, 0.1`, 0.029`, 0.062`, 0.058`,
   0.045`, 0.063`, 0.088`, 0.079`, 0.038`, 0.057`, 0.121`, 0.075`, 0.054`, 0.101`, 0.063`,
   0.069`, 0.069`, 0.046`, 0.047`, 0.113`, 0.058`, 0.094`, 0.09`, 0.067`};

ASmallSetOfKIvalues = {5.41`, 3.84`, 0.62`, 0.95`, 1.83`, 3.46`, 0.79`, 1.06`, 0.38`,
   4.06`, 4.1`, 3.54`, 0.61`, 2.85`, 3.2`, 3.82`, 1.45`, 1.3`, 1.25`, 0.96`, 3.63`, 0.43`,
   0.68`, 2.53`, 3.96`, 1.14`, 1.69`, 0.32`, 1.38`, 0.4`, 0.96`, 1.68`, 0.63`, 2.1`,
   0.34`, 1.79`, 0.15`, 15.78`, 0.2`, 0.12`, 0.42`, 1.90`, 26.7`, 0.24`, 0.89`, 1.25`,
   4.62`, 0.93`, 0.8`, 2.62`};

Do[
 ProgressionTimeCytotoxic[ASmallSetOfKIvalues[[SimulatedTumorNumber]], 1000,
  ASmallSetOfGrowthRates[[SimulatedTumorNumber]], ProgressionThreshold];
 TotalPopulationOverTimeCytotoxic[SimulatedTumorNumber] =
  Table[
   {t,
    ((p[t] /. mysimresultS) + (p[t] /. mysimresultR1) + (p[t] /. mysimresultR2) +
        (p[t] /. mysimresultRR)) / 10^10 - 1}, {t, 0, (SimulationDuration - 0.5) * 365, 10}];
 SPopulationOverTimeCytotoxic[SimulatedTumorNumber] =
  Table[{t, (p[t] /. mysimresultS) / 10^10 - 1}, {t, 0, (SimulationDuration - 0.5) * 365, 10}];
 R1PopulationOverTimeCytotoxic[SimulatedTumorNumber] =
  Table[{t, (p[t] /. mysimresultR1) / 10^10 - 1}, {t, 0, (SimulationDuration - 0.5) * 365, 10}];
 R2PopulationOverTimeCytotoxic[SimulatedTumorNumber] =
  Table[{t, (p[t] /. mysimresultR2) / 10^10 - 1}, {t, 0, (SimulationDuration - 0.5) * 365, 10}];
 RRPopulationOverTimeCytotoxic[SimulatedTumorNumber] =
  Table[{t, (p[t] /. mysimresultRR) / 10^10 - 1}, {t, 0, (SimulationDuration - 0.5) * 365, 10}];

 , {SimulatedTumorNumber, 1, 50}]

(* a subset was selected (non-randomly) to illustrate the diversity of different
 treatment outcomes *)
ListPlot[Table[TotalPopulationOverTimeCytotoxic[stn], {stn, {1, 3, 4, 7, 9, 10, 20, 22, 23}}],
 PlotRange → {{0, 30 * 30.5}, {-1, 1}}, Joined → True,
 PlotStyle →
  Table[Directive[Blend[{ColorData[16, i], ColorConvert[ColorData[16, i], "Grayscale"]}, 0.7],
    Opacity[1], AbsoluteThickness[2]], {i, {1, 2, 3, 4, 6, 7, 8, 9, 10}}],
 Frame → {{True, False}, {True, False}}, Axes → {False, False}, Ticks → None,
 PlotRangePadding → {{0, 0}, {0.0, 0}}, FrameStyle → Directive[Black, Thickness[Medium]],
 AxesStyle -> Directive[Black, Thickness[Medium]],
 BaseStyle → {FontFamily → "Arial", FontSize → 12},
 FrameTicks → {{Table[{i, 100 * i, {0, 0.02}}, {i, -1, 1, 1 / 2}], None},
   {Table[{i, i * 2 / 61, {0, 0.02}}, {i, 0, 2000, 6 / 4 * 122}], None}}, AspectRatio → 2 / 3,
 ImageSize → {{1000}, {200}}, ImagePadding → {{100, 10}, {60, 20}},
 FrameLabel → {"Time (months)", "Change from baseline\n tumor volume(%)"},
 Prolog → {Dashing[{0.015, 0.01}], Opacity[0.7], GrayLevel[0.], Thickness[Medium](*,
   Line[{{0,0},{2*366,0}}]*)}]

Export[NotebookDirectory[] <> "Supplementary Figure S7B, Cytotoxic model kinetics.pdf", %, "PDF"];
```



A larger batch produced by randomly drawn parameters

```
Do[
 ProgressionTimeCytotoxic[logKI1[[SimulatedTumorNumber]], 1000,
  DistributionOfGrowthRates[[SimulatedTumorNumber]], ProgressionThreshold];
 TotalPopulationOverTimeCytotoxic[SimulatedTumorNumber] =
  Table[
   {t,
    ((p[t] /. mysimresultS) + (p[t] /. mysimresultR1) + (p[t] /. mysimresultR2) +
        (p[t] /. mysimresultRR)) / 10^10 - 1}, {t, 0, (SimulationDuration - 0.5) * 365, 10}];
SPopulationOverTimeCytotoxic[SimulatedTumorNumber] =
 Table[{t, (p[t] /. mysimresultS) / 10^10 - 1}, {t, 0, (SimulationDuration - 0.5) * 365, 10}];
R1PopulationOverTimeCytotoxic[SimulatedTumorNumber] =
 Table[{t, (p[t] /. mysimresultR1) / 10^10 - 1}, {t, 0, (SimulationDuration - 0.5) * 365, 10}];
R2PopulationOverTimeCytotoxic[SimulatedTumorNumber] =
 Table[{t, (p[t] /. mysimresultR2) / 10^10 - 1}, {t, 0, (SimulationDuration - 0.5) * 365, 10}];
RRPopulationOverTimeCytotoxic[SimulatedTumorNumber] =
 Table[{t, (p[t] /. mysimresultRR) / 10^10 - 1}, {t, 0, (SimulationDuration - 0.5) * 365, 10}];

, {SimulatedTumorNumber, 1, 200}]
```

```
ListPlot[Table[TotalPopulationOverTimeCytotoxic[SimulatedTumorNumber],
  {SimulatedTumorNumber, 50, 100}], PlotRange → {{0, 3 * 366}, {-1, 1}}, Joined → True,
 PlotStyle → Table[Directive[Hue[RandomReal[{0, 1}], 0.7, RandomReal[{0.3, 0.5}]],
    Opacity[0.7], Thickness[Medium]], {200}], Frame → {{True, False}, {True, False}},
 Axes → {False, False}, Ticks → None, PlotRangePadding → None,
 FrameStyle → Directive[Black, Thickness[Medium]],
 AxesStyle -> Directive[Black, Thickness[Medium]],
 BaseStyle → {FontFamily → "Arial", FontSize → 12},
 FrameTicks → {{Table[{i, 100 * i, {0, 0.01}}, {i, -1, 1, 1 / 2}], None},
   {Table[{i, i * 2 / 61, {0, 0.01}}, {i, 0, 2000, 3 / 2 * 122}], None}}, AspectRatio → 1 / 2,
 ImageSize → {{400}, {500}}, ImagePadding → {{100, 10}, {60, 20}},
 FrameLabel → {"Time (months)", "Change from baseline\n tumor volume(%)"},
 Prolog → {Dashing[{0.015, 0.01}], Opacity[0.7], GrayLevel[0.], Thickness[Medium],
   Line[{{0, 0}, {3 * 366, 0}}]}]
```



This interface allows the user to scroll through different individual tumors and inspect the growth (or inhibition) of its sub-populations over time.

```
Manipulate[
 ListPlot[{TotalPopulationOverTimeCytotoxic[sample], SPopulationOverTimeCytotoxic[sample],
   R1PopulationOverTimeCytotoxic[sample], R2PopulationOverTimeCytotoxic[sample],
   RRPopulationOverTimeCytotoxic[sample]}, PlotRange → {{0, 30 * 30.5}, {-1, 1}},
  Joined → True,
  PlotStyle → Join[{Directive[Black, Opacity[1], AbsoluteThickness[3]]},
    Table[Directive[col, Opacity[1], AbsoluteThickness[2]],
     {col, {Darker[Green, 0.3], Red, Blue, Purple}}]], Frame → {{True, False}, {True, False}},
  Axes → {False, False}, Ticks → None, PlotRangePadding → {{0, 0}, {0.0, 0}},
  FrameStyle → Directive[Black, Thickness[Medium]],
  AxesStyle -> Directive[Black, Thickness[Medium]],
  BaseStyle → {FontFamily → "Arial", FontSize → 12},
  FrameTicks → {{Table[{i, 100 * i, {0, 0.01}}, {i, -1, 1, 1 / 2}], None},
    {Table[{i, i * 2 / 61, {0, 0.01}}, {i, 0, 2000, 3 / 4 * 122}], None}}, AspectRatio → 1 / 2,
  ImageSize → {{1000}, {300}}, ImagePadding → {{100, 10}, {60, 20}},
  FrameLabel → {"Time (months)", "Change from baseline (%)"},
  Prolog → {Dashing[{0.015, 0.01}], Opacity[0.7], GrayLevel[0.], Thickness[Medium],
    Line[{{0, 0}, {3 * 366, 0}}]},
  PlotLegends → {"Total population", "Most sensitive (S)", "Resistant to drug 1 (R1)",
    "Resistant to drug 2 (R2)", "Cross-resistant (RR)"}]
, {sample, 1, 200, 1}]
```