

Drug Combination Analysis

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abstract: Drug-combination analysis is discussed and demonstrated.

Suppose we have a set of drugs (or other treatments which we misclassify as “drugs” for the purpose of our analysis.) These drugs are supposed to be treatments for some disease, *e.g.*, HIV infection. Our goal is to assess whether, and to what extent, treatment with a particular combination of these drugs is more or less effective than some other such combination treatment. Such combination treatments include the case of treatment with a single drug, so we can also compare the efficacy of two distinct single drugs with our method.

For each treatment with a combination of drugs D_1, \dots, D_k , the data we have is a set of $k + 1$ dimensional points of the form (d_1, \dots, d_k, y) , where d_j is the dose of drug D_j (measured in any desirable units) and y is the *response* observed in the subject represented by the point at hand.

The observed response is always non-negative, and is generally a value in the interval $[0, 1]$. Such a value y denotes the relative amount or level of disease present *after* the drug treatment. Thus a small response value indicates a better response than does a larger response value. Choosing a reasonable and informative response measure is vitally important. A direct measure such as the amount of virus present is generally preferable, (although because this is biology, there are many unknowns and pitfalls in nearly every measure.)

Crudely, a response value y should be measured such that $100(1-y)$ is the percent improvement observed. For example, let w_0 denote a subject’s white blood cell count before treatment and let w_1 denote that subject’s white

blood cell count after treatment; then the response for that subject might be $1 - (w_1 - w_0)/w_1 = w_0/w_1$ which is 1 minus the relative improvement observed. The value 1 or greater denotes no suppression of disease, and values near 0 indicate substantial suppression of disease. Note $w_0/w_1 \in [0, \infty]$. We should try to avoid very large response values since the associated error is hard to characterize. A “personalized” response can be constructed as follows. Define a conservative interval $[w_l, w_h]$ for each subject in which every observed count must lie, and then take $1 - (w_1 - w_0)/(w_h - w_l)$ as the response for the subject whose pre- and post-treatment white cell counts are w_0 and w_1 respectively. Note $0 \leq 1 - (w_1 - w_0)/(w_h - w_l) \leq 1$.

Another example is that where we measure a quantity that is directly related to the level of disease present, such as the concentration of PSA present, normalized to lie in the interval $[0, 1]$, although here, as in most cases, a percentage response is more appropriate, since the amount of disease present prior to treatment can vary greatly.

For a single drug, a so-called logistic dose-response curve often provides an adequate description of the effect of treatment with that drug. *We assume that such a model for single drugs is appropriate here.* (Although, see the remarks below on a non-parametric approach.) The general logistic dose-response function for a single drug is:

$$y(d) = (a - h)/(1 + (d/c)^b) + h.$$

Here $y(d)$ is the response to treatment with a drug-dose of size d (remember a smaller response value is better than a larger response value.) The parameter a is the predicted response to a 0-dose (presumably, this response indicates no suppression of disease.) The parameter h is the predicted response to an infinite drug dose (ignoring toxicity and other practical issues.) The parameter c is the “mid-effect” dose, which is that dose that yields the response $(a + h)/2$. Often c is called the IC_{50} dose and is denoted by the symbol PC_{50} . The parameter b is called the slope parameter; it controls the shape of the dose-response curve defined by the function y .

In our case, we assume that $a = 1$ (*i.e.*, we have no suppression of disease at 0 dose) and that $h = 0$ (*i.e.*, we approach complete suppression of disease as the dose becomes sufficiently large.) Thus, $y(d) = 1/(1 + (d/c)^b)$. This dose-response curve generally “decays” sigmoidally from the value 1 to the value 0 as the dose d increases. If $b > 1$, we have faster decay, if $0 < b < 1$, we have slower decay, if $b = 0$, $y(d)$ is the constant value .5, and if $b < 0$, our dose-response curve *increases* instead of decaying.

We may construct a single drug dose-response model for given dose-response data $(d_1, v_1), \dots, (d_m, v_m)$ by estimating the parameters c and b that fit the model function y to our data via weighted least-squares minimization, (although no weights are used in the example below; this is essentially assuming the variances of the errors in each response observation are identical.)

An example showing the use of the MLAB mathematical and statistical modeling system[1] to read-in the dose-response data, define the single-drug logistic dose-response model function, provide initial guesses for the parameters c and b , fit our model to the data to obtain the least-squares estimates of c and b , and finally, to graph the results, is given below. (Often it is necessary to impose constraints on the parameters c and b in order to get a successful fit. This can be done in MLAB, but it is not needed in the example given here.)

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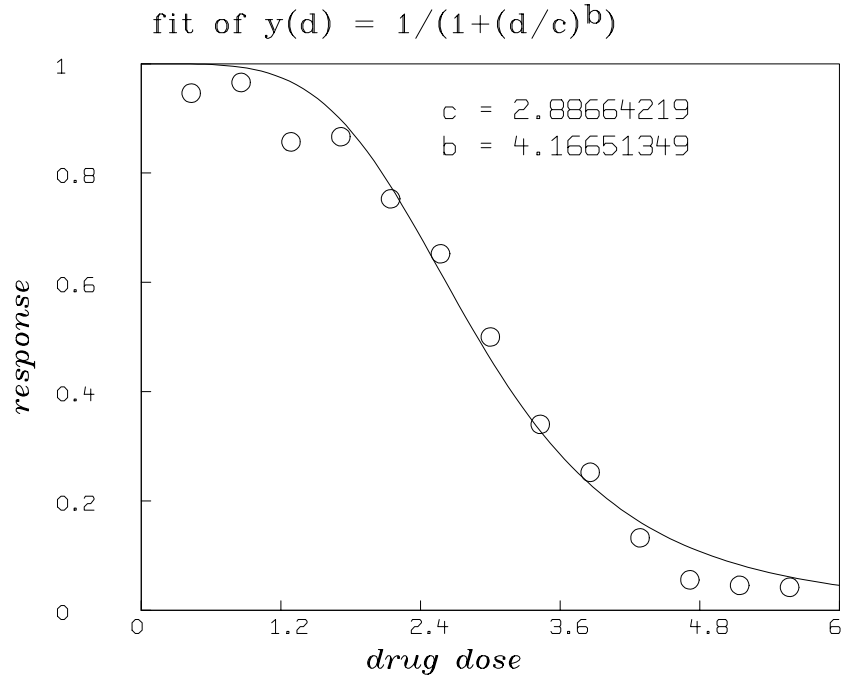
* dv=read(d1data,100,2)
* fct y(d) = 1/(1+(d/c)^b)
* c = 3; b = 1

* fit (b,c), y to dv
final parameter values
      value          error          dependency      parameter
      4.166513485      0.3632194177      0.05378619542      B
      2.886642188      0.06692784189      0.05378619542      C
6 iterations
CONVERGED
best weighted sum of squares = 2.664797e-02
weighted root mean square error = 4.921934e-02
weighted deviation fraction = 5.824418e-02
R squared = 9.833524e-01

* draw points(y,0:6!100)
* draw dv lt none pt circle ptsize .02
* left title "response" font 18
* bottom title "drug dose" font 18
* top title "fit of y(d) = 1/(1+(d/c)'.5ub'.5d)" font 7
* title "c = "+c at (.5,.8)
* title "b = "+b at (.5,.75)

```

* view



Now let us consider a two-drug combination treatment. The dose-response model in this situation is a function of two arguments, $y(d_1, d_2)$, that predicts the response to the combination dose (d_1, d_2) . Let us suppose that drug D_1 and drug D_2 have no interaction. Moreover, we postulate that $y(d_1, 0) = 1/(1 + (d_1/c_1)^{b_1})$ and $y(0, d_2) = 1/(1 + (d_2/c_2)^{b_2})$. We assume that the parameters c_1 , b_1 , c_2 , and b_2 are known due to fitting the single-drug logistic dose-response model to given single-drug dose-response data for drug D_1 and separately for drug D_2 . In analogy to the terminology used with multivariate distribution functions, we may call the single-drug logistic functions $y(d_1, 0)$ and $y(0, d_2)$ the *marginal* dose-response functions for the two-argument dose-response function y .

Let δ_1 be the value such that our non-interaction two-drug dose-response model satisfies $y(\delta_1, 0) = r$, and let δ_2 be the value such that $y(0, \delta_2) = r$. Now based on our no-interaction hypothesis, we can follow Bunow and Weinstein [2], and geometrically define $y(d_1, d_2)$ by postulating that $y(d_1, d_2) = r$ for (d_1, d_2) on the line-segment connecting $(\delta_1, 0)$ and $(0, \delta_2)$. Note it would

not be appropriate to define $y(d_1, d_2) = y(d_1, 0) + y(0, d_2)$ since drug D_1 and drug D_2 *compete* to suppress the disease, even if they act independently; that is, when drug D_1 has acted to suppress some of the disease, there is less “disease” present for drug D_2 to apply to, and conversely. The line-segment connecting $(\delta_1, 0)$ and $(0, \delta_2)$ is $L_r := \{(d_1, d_2) \mid (d_1, d_2) = \alpha(\delta_1, 0) + (1 - \alpha)(0, \delta_2), 0 \leq \alpha \leq 1\}$, and we specify that $y(d_1, d_2) = r$ for $(d_1, d_2) \in L_r$. This is appropriate because, when drug D_2 is the same as drug D_1 , the predicted response to a combination dose (d_1, d_2) *must* be the same as the predicted response to a dose of size $d_1 + d_2$ of drug D_1 (or D_2) alone! Any drug-interaction model which fails to satisfy this condition cannot be a statistically-correct model.

Now note that, if $z = 1/(1 + (d/c)^b)$, then $1 = (\frac{1}{z} - 1)^{-1/b}(d/c)$.

Recall that $y(\delta_1, 0) = r = 1/(1 + (\delta_1/c_1)^{b_1})$. Thus $1 = (\frac{1}{r} - 1)^{-1/b_1}(\delta_1/c_1)$, so $\alpha = (\frac{1}{r} - 1)^{-1/b_1}(\alpha\delta_1/c_1)$. Also $y(0, \delta_2) = r = 1/(1 + (\delta_2/c_2)^{b_2})$, so $1 = (\frac{1}{r} - 1)^{-1/b_2}(\delta_2/c_2)$, and thus $1 - \alpha = (\frac{1}{r} - 1)^{-1/b_2}((1 - \alpha)\delta_2/c_2)$.

But then, when $(d_1, d_2) \in L_r$, $d_1 = \alpha\delta_1$ and $d_2 = (1 - \alpha)\delta_2$ for some value $\alpha \in [0, 1]$, and we have specified that $y(d_1, d_2) = r$. Next, note that

$$1 = \left(\frac{1}{r} - 1\right)^{-1/b_1}(\alpha\delta_1/c_1) + \left(\frac{1}{r} - 1\right)^{-1/b_2}((1 - \alpha)\delta_2/c_2).$$

Therefore $y(d_1, d_2)$ can be defined as the value z such that $(\frac{1}{z} - 1)^{-1/b_1}(d_1/c_1) + (\frac{1}{z} - 1)^{-1/b_2}(d_2/c_2) = 1$ for any non-negative values of d_1 and d_2 . Note this construction insures that $y(d_1, d_2) = r$ for $(d_1, d_2) \in L_r$.

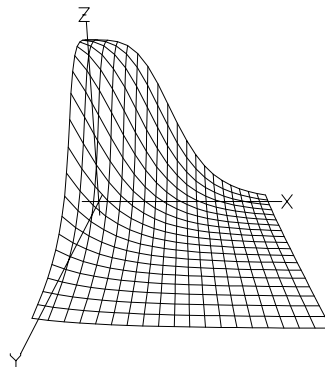
This Bunow-Weinstein two-argument dose-response function for non-interacting drugs is a logically-correct generalization of a single-drug logistic dose-response function. This implicit function can be defined in MLAB as shown below. Note the care that is taken to avoid division by zero and raising zero to a negative power.

```
function y1(d1)=1/(1+(d1/c1)^b1)
function y2(d2)=1/(1+(d2/c2)^b2)

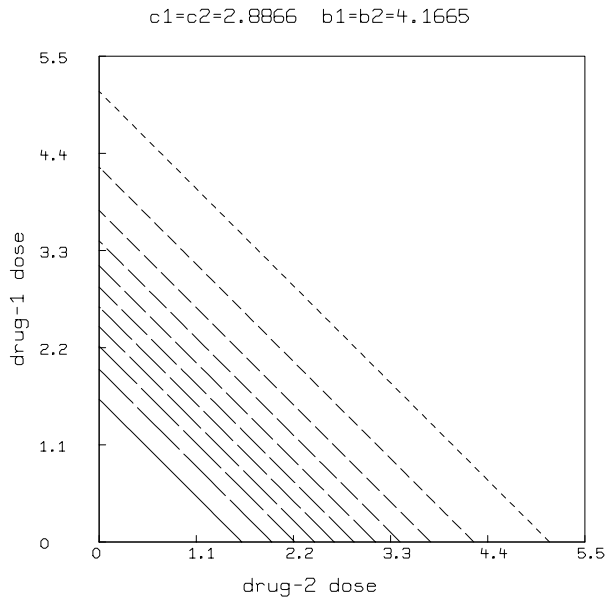
function y(d1,d2)=if d1=0 then y2(d2) else \
                  if d2=0 then y1(d1) else \
                  if y1(d1)<.00001 and y2(d2)<.00001 then 0 else \
                  if y1(d1)>.99990 and y2(d2)>.99990 then 1 else \
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$$\text{root}(z, 1e-11, 1-1e-11, (d1/c1)*(1/z-1)^{-1/b1} + (d2/c2)*(1/z-1)^{-1/b2} - 1)$$

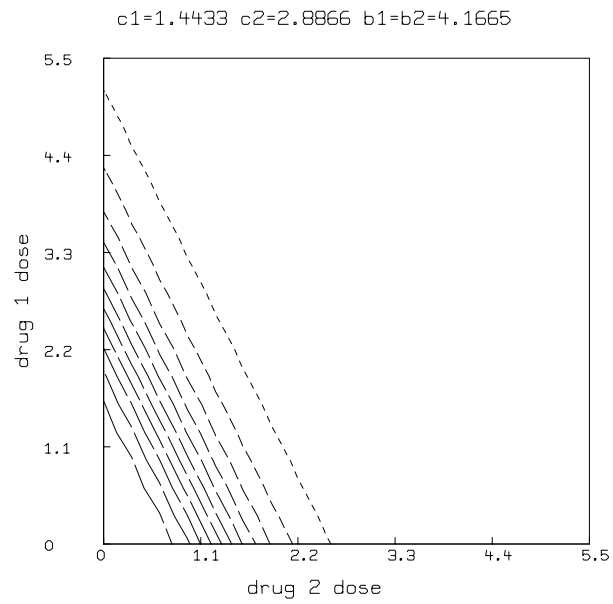
MLAB was used to produce a picture of the “response-surface” defined by our model function y for two non-interacting drugs with identical marginal curves defined by $c_1 = c_2 = 4.1665$ and $b_1 = b_2 = 2.8866$ which is given below.



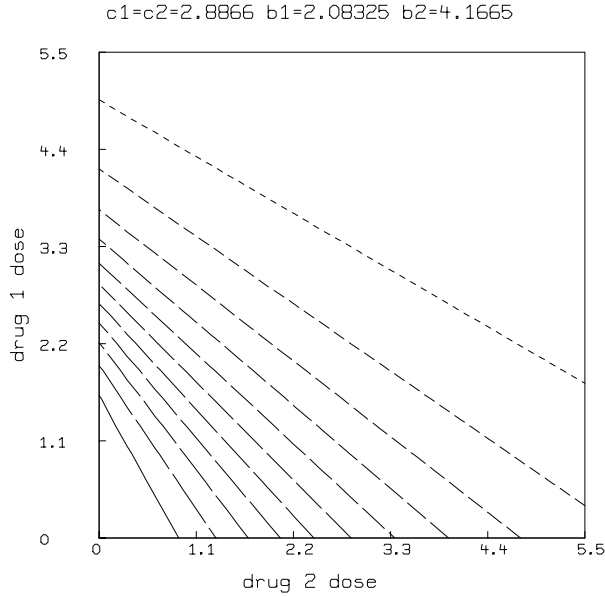
It is also useful to see the contour map corresponding to the function y with $c_1 = c_2 = 4.1665$ and $b_1 = b_2 = 2.8866$ corresponding to the surface shown above. The contour map shows some level lines where $y(d_1, d_2)$ is constant.



Here is the contour map corresponding to the function y in the case where $c_1 = 2.08325$, $c_2 = 4.1665$ and $b_1 = b_2 = 2.8866$.



Finally, here is the contour map corresponding to the function y in the case where $c_1 = c_2 = 4.1665$, $b_1 = 1.4433$ and $b_2 = 2.8866$.



Note that the Bunow-Weinstein non-interacting two-drug dose-response function can be immediately generalized to the case of k non-interacting drugs. In general we have:

$$y(d_1, \dots, d_k) = \text{root}_{0 \leq z \leq 1} [-1 + \sum_{i=1}^k (\frac{1}{z} - 1)^{-1/b_i} (d_i/c_i)].$$

Now we may consider drug combination treatments where the drugs involved may interact synergistically or antagonistically. To analyze such treatments, we need to have estimated the parameters $c_1, b_1, \dots, c_k, b_k$ appearing in the marginal single-drug dose-response functions for the k drugs under consideration, so that the Bunow-Weinstein non-interaction model is specified.

Given the data $(d_{i1}, d_{i2}, \dots, d_{ik}, v_i)$ for $i = 1, \dots, m$, we may compute the predicted *non-interaction* response values $r_i = y(d_{i1}, d_{i2}, \dots, d_{ik})$. If we have no interaction, the values r_i should be approximately the same as the observed response value v_i . If not, then we can conclude there is evidence for a synergistic or antagonistic effect, and the size and direction of this

effect can be estimated from the response differences $v_i - r_i$. (Note that for the same combination of drugs, we might have *both* synergistic effects for dose-pairs in some regions and antagonistic effects in different dose-pair regions.)

It may be convenient to consider the fractional differences $p_i := 1 - v_i/r_i$; $100p_i$ is the percent change from the non-interaction predicted response r_i that is observed in the actual response v_i . If $v_i < r_i$, the disease was suppressed more than the non-interaction model would predict and $p_i > 0$. If $v_i > r_i$, the disease was suppressed less than the non-interaction model would predict and $p_i < 0$. Crudely, we might say that our drug-combination exhibits a $100(p_1 + \dots + p_m)/m$ percent synergy interaction effect.

It is interesting to plot the p_i -values, the v_i -values and/or the r_i -values versus the associated *equivalent single-drug-dose* of drug D_1 (or any other of the drugs involved) as determined by the Bunow-Weinstein non-interaction model y . If (d_1, \dots, d_k) is the vector of dose values corresponding to the data-value v_i and the predicted non-interaction value r_i , then the equivalent drug D_1 dose is the value e such that $y(e, 0, \dots, 0) = r_i$; An MLAB function involving the root-operator can be defined to compute such equivalent dose values, but e can be directly computed as $c_1/(\frac{1}{r_i} - 1)^{-1/b_1}$ (with due care for $r_i = 0$ and for $r_i = 1$.) Such plots may be useful in seeing how the synergy effect varies with dose.

Now one way to decide if the r_i -values are, over all, sufficiently greater than the v_i values to conclude that we have statistically-significant synergy is to test the hypothesis that the mean of the p_i -values is less than or equal to 0 versus the alternate hypothesis that the mean of the p_i -values is greater than 0. A similar test can be used to assess whether we have statistically-significant antagonism.

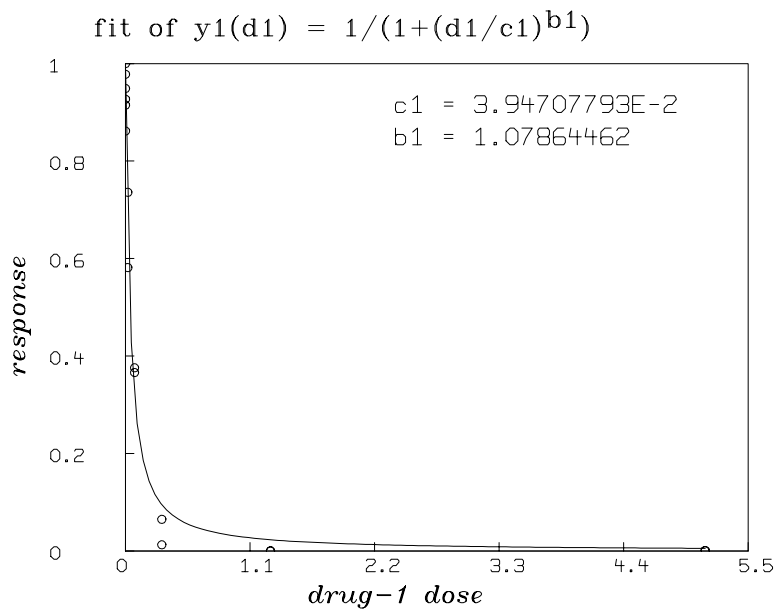
It is probably most appropriate to use a non-parametric test such as the Wilcoxon 2-sample paired-data test in MLAB on the r_i -values versus the v_i -values. And we can check our outcome with a paired-data t -test on the p_i -values, even though the normal-distribution assumption used there is invalid.

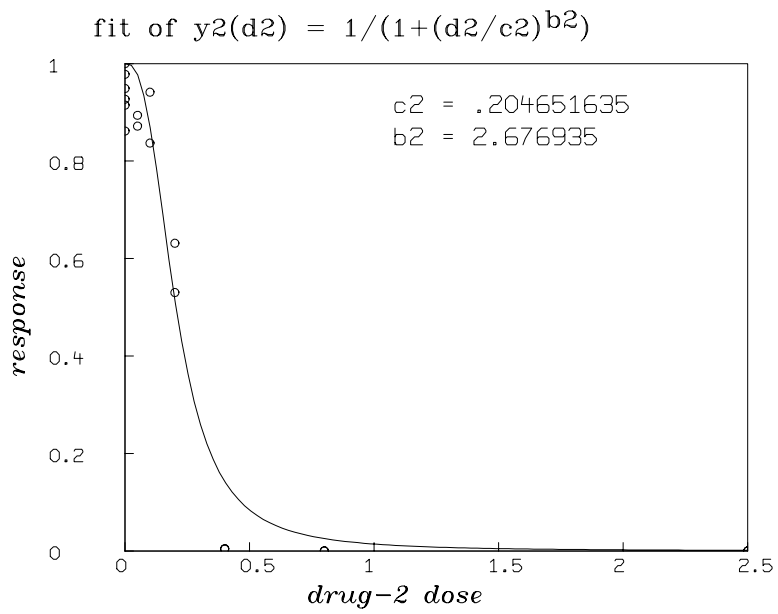
Note that in order to decide which of two drug combination treatments is better we need only compare the p_i -values for treatment 1 with the p_i -values for treatment 2.

Let us look at an example of the results of using MLAB to perform

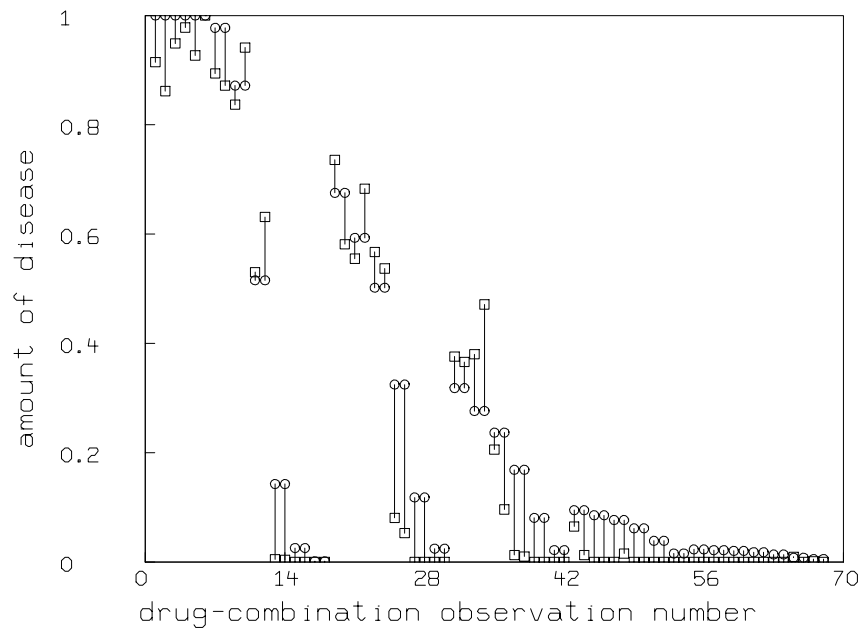
an analysis of some two drug combination responses at various doses. We have a file called “a3azt.in” which contains n lines of three numbers, where the first number is the administered dose of drug D_1 , the second number is the administered dose of drug D_2 , and the third number is the response measured as the amount of disease present after treatment (in this case, the response is the amount of virus assayed.) Each line in our data file is thus an “observation” from a single “experiment”.

In order to produce the graphs given below we used MLAB to read our data, normalize the response values v_1, \dots, v_n to lie in $[0, 1]$, extract the data for the two marginal dose-response curves (one data-set where the drug D_1 dose is 0 and one data-set where the drug D_2 dose is 0), fit to obtain the estimated marginal dose-response curve parameters $c_1, b_1, c_2,$ and b_2 (which then define our Bunow-Weinstein non-interaction two-drug dose-response function), compute the predicted non-interaction response values r_1, \dots, r_n , and then compare the predicted r_i -values with the observed v_i -values to study the nature and amount of synergism apparent between our two drugs.

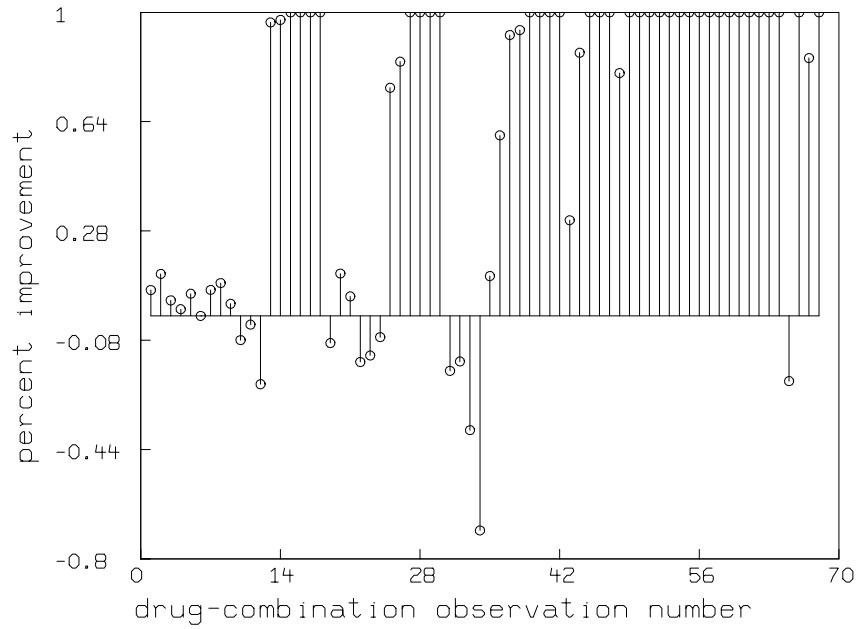


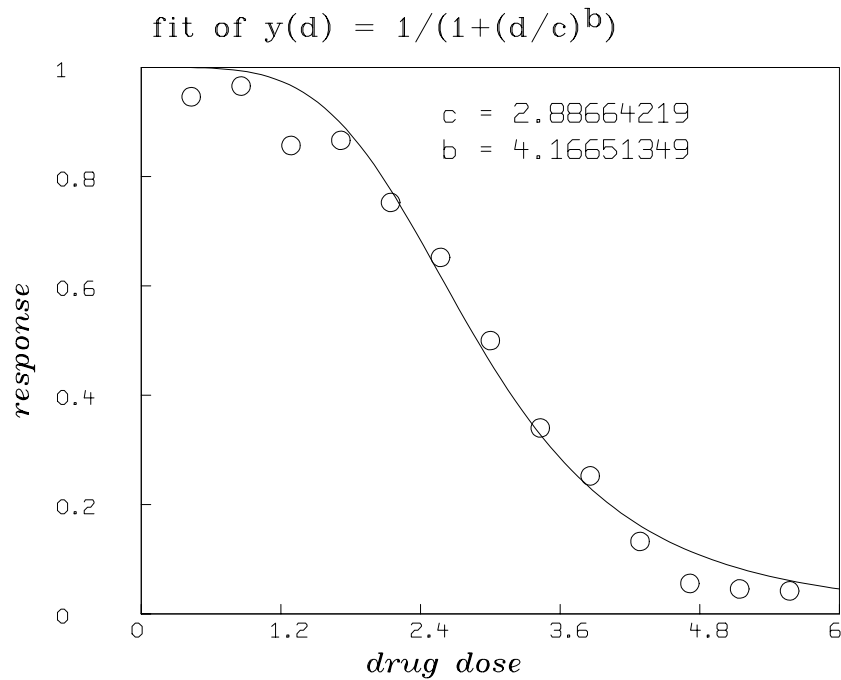


squares:data, circles:non-interaction prediction



fractional change from non-interaction response





We used the Wilcoxon 2-sample signed-ranks test for paired data in MLAB to compare our data v with the corresponding predicted non-interaction response values r . The null hypothesis is $median(v) = median(r)$. In our case, the absolute sum of negative ranks was 1819. Let W denote the Wilcoxon test statistic. The probability $P(W > 1819) = .000011$. This means that a value of W at least as large as 1819 arises about 0.001050 percent of the time, given the null hypothesis.

Since the probability $P(W > 1819)$ is small, we can reject the null hypothesis of equal responses, with probability .99989 of being correct, and we can conclude that the alternate hypothesis: $median(v) < median(r)$ probably holds, so we accept that synergism is present.

Note that the graphs presented above can be constructed and have interest in the more-general situations of more than two drugs being used in combination.

When we have synergy, we will generally see that the bulk of the observed v_i -values, plotted with respect to the equivalent dose e_i of one of the drugs, lie *below* the marginal dose-response curve for that drug. This occurs

above, where we compare the marginal curve for drug D_2 with our observed response vs. D_2 -equivalent dose amounts. We may fit a single-drug dose-response model to these (e_i, v_i) points; the difference between this fitted curve and the single-drug marginal curve is a suitable basis for computing the degree of synergism or antagonism at that equivalent dose.

Sometimes we may have the situation where a drug D_2 has no therapeutic effect, but may, nevertheless, modify the effect of another drug D_1 when used in combination. In this case, we should define the Bunow-Weinstein non-interaction model function so that $y(d_1, d_2) = y(d_1, 0)$ (i.e., $y(d_1, d_2) = 1/(1 + (d_1/c_1)^{b_1})$.) If this model does *not* fit our data, then we have statistically demonstrated the pure potentiation or inhibition behavior of drug D_2 on drug D_1 , where drug D_2 , by itself, has no effect.

It is important to note that to use the approach to drug-combination analysis presented here, we must have the marginal single-drug dose-response curves specified by some means. If this is not possible, we can use a direct model-based approach due to Bunow and Weinstein. This approach requires that we fit an explicit model function to our drug combination data, where the model function contains explicit interaction terms. If the estimated interaction parameters are significantly different from their “no-interaction” value, then we have evidence for synergy or antagonism (but not both in different regions.)

One such drug-interaction model proposed by Bunow and Weinstein is:

$$y(d_1, d_2) = \text{root}_{0 \leq z \leq 1} \left[-1 + \left(\frac{1}{z} - 1 \right)^{-1/b_1} (d_1/c_1) (1 + a_{12} d_2^{e_{12}}) + \left(\frac{1}{z} - 1 \right)^{-1/b_2} (d_2/c_2) \right].$$

In this model, the term $a_{12} d_2^{e_{12}}$ determines an change in the efficacy of drug D_1 in the presence of drug D_2 , which itself has an independent effect. When a_{12} is 0, there is no interaction. The greater a_{12} and e_{12} are, the greater is the dose-dependent potentiation effect of drug D_2 on drug D_1 . When a_{12} is negative, drug D_2 has an inhibitory effect on drug D_1 . Fitting such a model requires some care in the curve-fitting process. Numerical issues such as division by zero and zero raised to a negative power must be handled. Generally, appropriate weights are required, and often good initial parameter guesses and accompanying constraints are also needed. For example, it may be appropriate to constrain e_{12} to be non-negative. MLAB can accept such weights and constraints, and with some care, can be used to estimate the desired parameters.

It is tempting to suppose that the estimated values of c_1 and b_1 determine the drug D_1 single-drug dose response curve, and likewise for c_2 and b_2 , but this is an unwarranted assumption. What we do know is that if a_{12} is significantly different than 0 and e_{12} is not a large negative value, then our data prefers to follow this interaction model, rather than the pure non-interaction model, and we take this as evidence of interaction. The basic underlying assumption is still that the actions of drug D_1 and drug D_2 separately are well-described by logistic dose-response curves as discussed above.

The entire drug-combination analysis method presented here can be recast in a more-general non-parametric framework. We can use a non-parametric estimate for each of our single-drug marginal dose-response curves, instead of using the logistic model. The Bunow-Weinstein non-interaction model can be defined with respect to these marginals, and the subsequent analysis proceeds as before. A suitable non-parametric estimate of a single-drug dose-response curve can be obtained in MLAB by first using the function MONOT to *monotonize* the data, and then using the function SMOOTHSPLINE to obtain an estimating optimal smoothing spline.

There is a serious practical problem with *any* drug-interaction analysis method which uses dose-response data as is done here. The problem is that at high (therapeutic-level) doses, for some types of measurement of the amount of disease present, the error in the response-values may be so magnified that such measurements may be predominantly noise, and yet this is likely to be the domain in which treatment will actually occur. Practically speaking, it is much more important that there be no serious antagonism present than that our drug-combination be synergistic; as long as the drugs involved are not antagonistic and are jointly effective, it is generally useful to use them in combination. Of course such possible antagonism can be assayed via the analysis methods discussed here, subject to the same caveat of high errors in the response data. You might imagine that if synergism is seen at moderate (IC_{50} -level) doses (where measurement error may be less problematical), then it will also be present at higher therapeutic doses, but this is not always the case. The conclusion is that when the drugs involved can be usefully administered at levels where our observed responses will be predominately noise because of the nearly complete suppression of disease expected, drug-combination analysis does not take the place of clinical trials using the drugs at therapeutic levels in order to assess the efficacy of the treatment.

[1] see www.civilized.com

[2] Bunow, Barry J. and Weinstein, John N. "Combo: A New Approach to the Analysis of Drug Combinations in Vitro", *Annals N.Y. Acad. of Science* Vol. 616, pp. 490-494, 1990.