

Using JMP[®] Software to Get a Jump on Childhood Leukemia by Modeling Assay Data

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Introduction

Treatment for childhood Leukemia patients has improved over the last twenty years. Increasingly, *in vitro* drug sensitivity results seem to provide important insight into inter-individual drug resistance (Klumper et al. 1996; Norgaard et al. 1996). These results appear to offer strong prognostic evidence which, when utilized to modify treatment, may improve outcome. However, the methodology associated with analyzing patients' assays is often crude. Linear interpolation (often visual) is used to calculate the concentration of a chemotherapeutic agent which provides a lethal dose to 50% of the cells. Thus, no confidence limits can be obtained about the parameter estimates of choice. Moreover, this method fails to consider:

- if the specimens being studied were proliferating or non-proliferating,
- more than one distinct response to the agent being tested,
- the steepness of the dose response curve, and
- the failure of the dose response curve to reach a lower limit of 0 or an upper limit other than 100%.

Modeling allows for better use of the assay data in that parameters of interest and their standard errors can be estimated. JMP[®] offers several features that facilitate the modeling process:

- The graphical interface allows trials of initial parameter values.
- The ability to "lock" and "unlock" parameters into fixed values enables convergence when there is difficulty.
- The profile option allows better estimates of confidence limits for the parameters.

Methods

Acute Myelogenic (AML) and T-cell patients were accrued from participating institutions of the Pediatric Oncology Group. All assays were set up utilizing a 96 well microculture plate with 5 wells used as controls. For each agent, including Daunorubicin (DNR), there were 18 wells in serial dilution. The percentage of viable cells was determined after 72 hours. To fit assay data, T-C-Chou's (Chou, 1994; Chou, 1991; Chou and Rideout, 1991; Chou and Talalay, 1987) median effects model for one population was generalized to allow for a mixture of two or more sub-populations.

The Hill median effects model is given by:

$$F = \frac{100}{[1 + D_m/D]^m}$$

where F will lie between 0 and 100, D is the dose and D_m is the dose required for the "median" effect at $F=50$. When $D = D_m$, m is a multiple of the slope (the first derivative) and the second derivative is zero, denoting a non-horizontal point of inflection. The parameter m is a Hill-type coefficient and signifies the sigmoidicity of the dose effect curve.

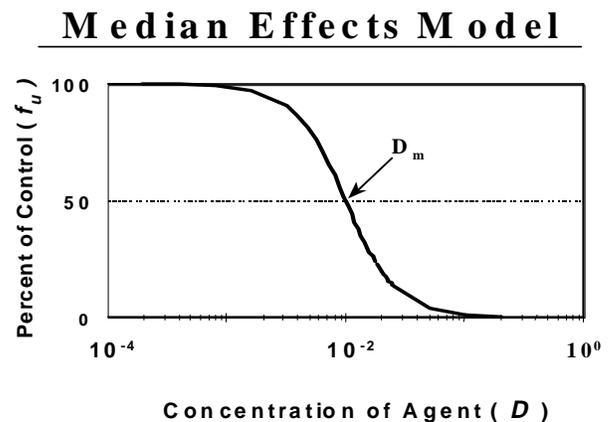


Figure 1.

Because of the proliferation of control cells, the maximum value possible may not be 100%, nor the minimum possible 0%. In this case, the median is not 50% and hence, the dose for which $F=50$, the LC50, may not be appropriate (Figure 2). The median effects (D_m), which is **not necessarily** the LC50 may be a more appropriate parameter to evaluate patients' responses.

Median Effects Model With Different Limits

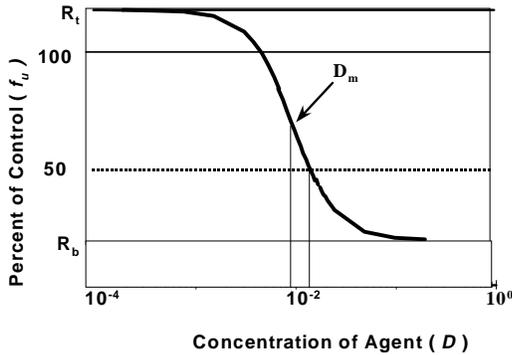


Figure 2.

To consider distinct subpopulations of cells in the leukemic specimens and to allow the fraction to have varying upper and lower limits, the Hill median effects model was modified:

$$F = (r_t - r_b) * \left[\frac{p_1}{1 + (D/D_{m1})^{m1}} + \dots + \frac{p_n}{1 + (D/D_{mn})^{mn}} \right] + r_b$$

Two Population Median Effects Model

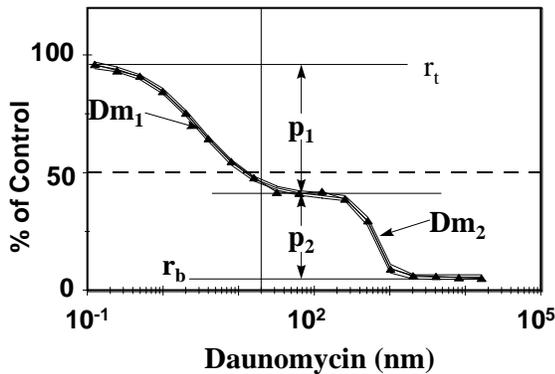


Figure 3.

Implementing the Model in JMP

To model the assay data in JMP® (JMP Statistics and Graphics Guide, 1995), a two-population model was used. Reparameterization of the equation was necessary to ensure constraints, such as the probabilities p_i lying between 0 and 1:

$$F = (r_t - r_b) * \left[\frac{p_1}{1 + (a_1 e^{(m_1 * \ln(D))})} + \frac{(1 - p_1)}{1 + (a_2 e^{(m_2 * \ln(D))})} \right] + r_b$$

Choosing appropriate starting values for parameters is important in obtaining reliable parameter estimates. One useful strategy is to estimate some parameters, while fixing the remaining parameters. Once this initial model has converged, the fixed parameters can be “unfixed” and the full model run. JMP’s parameter locking facility makes this process as easy as clicking a box. For this data, we found there was some instability in the estimation of some parameters with convergence occurring at unrealistic values, such as $r_t=350$. However, fixing r_t at values close to 100 and r_b at values near 0 convergence could be obtained, and then reasonable estimates for r_t and r_b obtained.

One feature JMP® does not have is the ability to set upper and lower parameter limits, beyond which the estimates are not interpretable. For example, it would be useful to set upper and lower constraints on r_t such that estimates could not go below 75 or above 125.

We found that with at most two subpopulations ($n=2$) we were able to fit the data adequately. In fact, p_2 was significantly different from 0 for most patients, indicating that the two-population model was necessary, as shown in Figure 4. The number of specimens achieving an improved fit varied with the agent being tested and the type of disease (refer to Table 1). Also, notice that The limits did not necessarily approach 100 and 0 as dictated by the unmodified model, and estimates for some patients were less precise as indicated by their confidence limits (Figure 5).

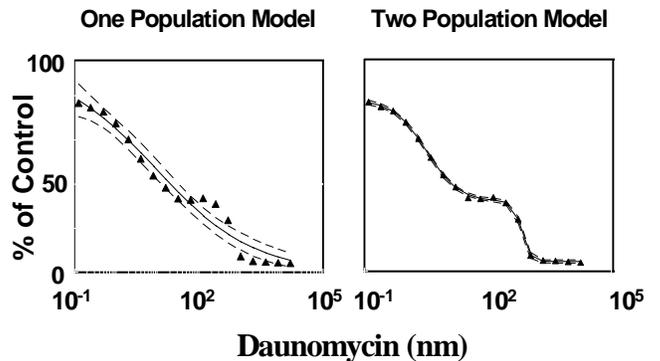


Figure 4.

Table 1. Improvement of Fitting Chemosensitivity Curves with a Two-Population Model

Daunomycin		Cytarabine	
AML	T-cell ALL	AML	T-cell ALL
28/29	9.21	15/29	12/20
(97%)	(43%)	(52%)	(60%)

p<0.05 utilizing an F-test for each chemosensitivity curve with an improved fit.

Future Study

We will need to develop models to fit more general assay situations where, for example, there are an unequal numbers of replicates and where we need estimates for the population rather than individual patients. We will probably take a non-linear mixed model approach (Ryan, 1992), rather than using the deterministic approach of non-linear modeling for each patient. It may be necessary to use SAS[®] rather than JMP[®] to do this, but it is likely that JMP[®] will still be useful for initial estimates. Ultimately, we are interested in using the modeling approach to find which aspects of assay best predict outcome of patients.

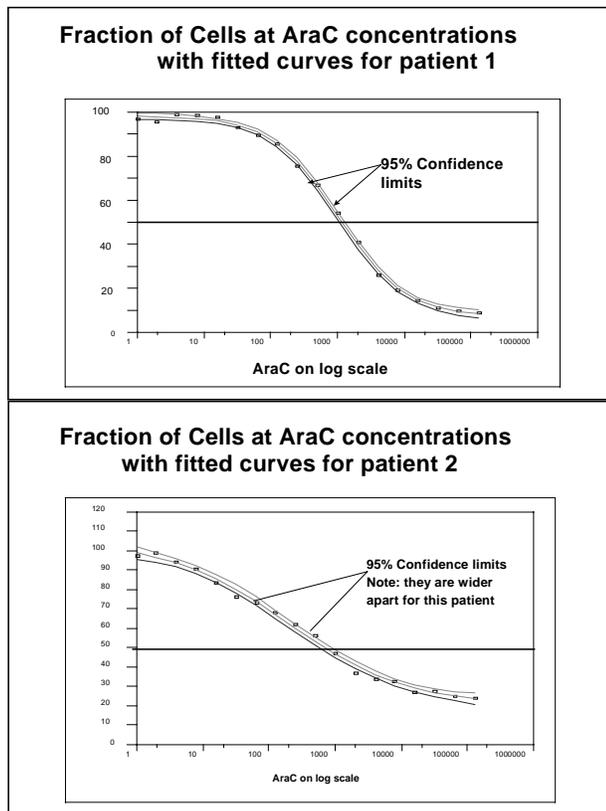


Figure 5.

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