A Bayesian Hierarchical Nonlinear Regression Model for Dose-Response Assessment and Synergy

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In toxicology and drug development, dose-response assessment and evaluation of interaction (e.g., synergism or antagonism) between combined agents are essential. For example, investigators are often interested in fitting dose-response curves and finding inhibitory concentrations, such as the IC25, IC50, and IC75, for a single compound alone and when combined with other agents in cell lines. Many dose-response experiments include replicate experiments, often under varying conditions. Challenge comes when trying to account for the many sources of variability inherent in the data.

In this paper, we propose a Bayesian hierarchical nonlinear regression model that allows estimating the inhibition concentrations while accounting for variation within and between experiments. We apply the proposed model and method to a motivating data set from an ovarian cancer research study. We illustrate the method with a study aimed at investigating four cancer therapy drugs, decitabine (DAC), azacitidine (AZA), suberoylanilide hydroxamic acid (SAHA), and trichostatin A (TSA) in two ovarian cancer cell lines, HEY and SKOV3. The experiment proceeds by manually pipeting cancer cells (e.g., HEY) into individual wells. The wells are grouped into sets of three wells allowing for three replicate experiments at each drug concentration. The study investigates ten dose levels including the control (concentration = 0). The effect of the drug concentration is monitored by optical density (OD) measurements, which characterize the number of cells surviving. For the most part, the in vitro data show a dose-response relationship for the investigating therapies in cell lines HEY and SKOV3. The objectives include fitting a dose-response curve, estimating inhibitory concentrations, and determining the interaction between combined therapies. We follow the usual practice and model the relationship between concentration (Conc) and response (Effect = OD) with the modified Hill's equation, also known as the Emax model (equation 1). Effect = Emax / [1 + (Conc/IC50)^(-m)] (Equation 1) Emax is the control effect or OD when Conc equals 0. This is a two-parameter nonlinear model.

The parameters of interest in the study are the IC50. IC50 is a common measure of drug effectiveness. It represents the concentration of the drug required for 50% inhibition of the cell line. Our goal is to estimate the IC50. To achieve this goal, we apply a Bayesian hierarchical model. In Bayesian analysis, we do not treat the parameters as fixed but as random. Our model approach considers that the individual experiments belong to a population of experiments. The hierarchical model allows us to account for different sources of variation (between-experiment, within-experiment but between replicates, and deviation of each replicate from the \\\"truth\\\"). In practice, this makes sense since you would expect there to be variability in the IC50 across experiments. In the following paragraph we introduce our proposed inferential model and describe our algorithm for fitting the model. We construct a model for each drug and cell line combination. Our tasks include (1) modeling the residual errors, and (2) setting priors and hyperpriors for the parameters (IC50, m, and Emax). We generally observe skewed, lognormal-like residuals. This is often due to biological parameters having physiological bounds, such as the effect being between 0 and Emax. Given this, we model the residual errors on the logarithmic scale i.e., the logarithm of the OD for any experiment is, on average, the logarithm of the right side of equation 1. Parameters (IC50, m, and Emax) are allowed to vary across drugs and experiments. The variability of the control effect, Emax, is due to the difficulty in manually pipeting equal amounts of cells into the wells from experiment to experiment. If this variability is not accounted for, dose-response assessment can be distorted. We model Emax as normally distributed and parameters m and IC50 as lognormal distributions with drug-specific mean values. We assign vague prior distributions to the parameters, so that the resulting posterior distributions will reflect the observed data. We make use of WinBUGS software to perform Markov chain Monte Carlo (MCMC). The simulation ran for 30,000 iterations, but we excluded the first 20,000 as burn-in. We obtain approximate convergence after 20,000 iterations. The MCMC output contains 10,000 samples from the posterior distribution of each random variable. Inferential

summaries of the dose-response curves consist of estimates of the inhibition concentrations, relative to experiment-specific control wells, leading to inhibition of 25% (IC25), 50% (IC50), and 75% of control (IC75). We characterize uncertainty of these estimates by their respective 2.5% and 97.5% quantiles, yielding an overall 95% credible interval. We plot the dose-response curves using the population median. We integrate results from single agent and combination studies to produce isobolograms. The isobolograms are based on Loewe's model of additivity (equation 2), which allows one to assess the presence of synergy or antagonism qualitatively when combining two or more agents. [drug1]comb/[drug1]alone + [drug2]comb/[drug2]alone = 1 (Equation 2) In the equation, [drug1]comb is the concentration of drug 1 in the combination that produced the given effect (Effect), and [drug1]alone is the estimated concentration of drug 1 alone that will produce the same effect (Effect).

When the combined agent's observed effect is more or less than predicted by Loewe additivity, we conclude Loewe synergism and Loewe antagonism, respectively. We plot isobolograms for each combined drug agent at each effect of interest. Two agents exhibit synergy when the isobologram is below and to the left of the line of additivity. If the line is above and to the right of the line of additivity, then the two agents are antagonistic for the effect at these concentrations. Assigning a descriptive name to an agent can be useful in determining if further studies are deserved.