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Letters to the Editor

I *Letter to the Editor* in response to the paper: Efficacy of ganciclovir in combination with zidovudine against cytomegalovirus in vitro and in vivo. Freitas, V.R., Fraser-Smith, E.B., Chiu, S., Michelson, S. and Schatzman, R.C. (1993) Antiviral Res. 21, 301–315.

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Freitas and co-workers at Syntex Research have reported recently that zidovudine (AZT) potentiates the antiviral activity of ganciclovir (DHPG) against a clinical isolate of human cytomegalovirus (HCMV) and interacts in an additive manner with a laboratory strain (AD169) of the same virus (Freitas et al., 1993). In contrast to our studies (Prichard et al., 1991) and those of others (Tian et al., 1991; Medina et al., 1992), Freitas and co-workers did not observe in vitro synergistic toxicity with AZT and DHPG using a new analytical method. A possible explanation for these differences may be attributable to the different experimental parameters selected. Freitas and associates assayed metabolic activity in mitochondria with MTT assays, both Tian et al. and Medina et al. assessed cytotoxicity by determining the number of viable cells (trypan blue exclusion) and total cellular protein (bicinchoninic acid method), whereas we chose to measure cytotoxicity by counting viable cells and by measuring the effects of drugs on plating efficiencies. In vitro studies aside, clinical experience has clearly revealed, however, that the concomitant administration of AZT and DHPG is contra-indicated (Jacobson et al., 1988; Hochster et al., 1990; Millar et al., 1990). A newly developed regimen does allow the co-administration of AZT and DHPG under certain controlled conditions (Causey, 1991). In this protocol, AZT may be given concomitantly with DHPG if the absolute granulocyte counts are above 750/μl. If the absolute granulocyte counts are initially below 750/μl or fall below 750/μl, AZT is discontinued, while DHPG is administered. This proto-

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col minimizes potential toxicity by avoiding concurrent dosing with AZT and DHPG when hematologic cytotoxicity becomes evident.

We are futher concerned by the misrepresentation of our analytical method (MacSynergy^{TM} II) by Freitas and co-workers. In their publication, the authors describe a step in our analysis as follows: "This theoretical additive surface is calculated based on a series of assumptions which must be made about additivity of the two drugs, and has to be predicted beyond the IC_{50} by using the equations in Prichard and Shipman's model. Even if one were to assume non-additive drug effects, the method still requires extrapolation. Such extrapolation will severely impact the adequacy of the analysis." They continue, "By avoiding the assumptions made by Prichard and Shipman (1990), as well as by Tian et al. (1991), the present analyses are necessarily more conservative and consequently reduce the false-positive rate." These statements are inaccurate in several respects and are related to important details missing from the Freitas et al. paper. The analysis of drug interactions attempts to characterize synergistic (greater than additive) or antagonistic (lesser than additive) effects. Therefore, "additive" effects must be defined by any analytical model including the new model described in the Freitas et al. offering. Two fundamental and non identical assumptions are commonly used to calculate theoretical additive effects (Loewe, 1953; Berenbaum, 1981; Chou and Talalay 1984; Greco et al., 1990; Greco et al., 1992; Prichard and Shipman, 1992). Our analytical method uses the "Bliss independence" assumption. This is the only assumption required to calculate theoretical additive effects that does not extrapolate any experimental data. Therefore, the different conclusions reached by our laboratory and the Syntex laboratory cannot be due to extrapolation as suggested by Freitas et al.

We are unable to fully evaluate the analytical model presented by Freitas et al. in as much as essential details are missing from the report. The statistical analysis is presented in minute detail, yet the fundamental assumption of additivity is not given. Their unstated assumption is important as the "Loewe additivity" assumption requires extrapolation and/or curve fitting and would affect the authors' conclusions if used. An additional troubling aspect of the Freitas et al. analytical model is that it seeks a "consistent deviation from additivity across the entire dose-response surface". Typically, synergy or antagonism are observed at a restricted subset of drug concentrations (for examples, see Lambert et al., 1993; Prichard et al., 1993). A statistic indicating the lack of a consistent response across the entire dose-response surface should not be interpreted as indicating that no synergism or antagonism exists. We trust that further studies are in progress to confirm the results produced by the Freitas et al. experimental model and that important details about this model will be forthcoming.

Based on their newly described analytical methods, Freitas and colleagues suggest that ganciclovir can safely be used with zidovudine for the treatment of opportunistic HCMV infection in AIDS patients. Our interpretation of the published preclinical and clinical literature and our own experimental data would strongly suggest that clinical investigators should proceed with caution when considering combinations of DHPG with licensed or experimental anti-HIV drugs.

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