## Reply

In reference to the correspondence from Drs. Samuels and Ratain, regarding the publication "Combined Use of Cyclosporin A and Verapamil in Modulating Multidrug Resistance in Human Leukemia Cell Lines" (1), we would like to make the following comments.

1. The mechanism of action of CyA[1] in modulating the multidrug-resistant phenotype is unclear. We and others have observed an alteration in the cellular pharmacokinetics of anthracyclines in cells exposed to CyA (2, 3). However, the mechanisms responsible for this alteration in drug kinetics are poorly understood, with some evidence suggesting that CyA and Vp differ mechanistically in their dependence on energy consumption. Thus, it is difficult to presuppose how these two agents might interact when used simultaneously to modulate the multidrug resistance phenotype.

2. Samuels and Ratain are critical of the use of the fractional product method used to calculate synergy in our original manuscript. However, the isobologram technique suggested by these authors is also imperfect and may provide misleading information regarding the synergistic interaction between any two reagents (4). Calculations used to analyze data in order to produce an isobologram often rely on the use of arbitrarily selected end points (e.g., the point at which growth is inhibited by 50%) which represent mathematical derivations of observed data (4). Thus, despite its limitations we have now used a third technique, the combination index method to estimate the interaction between CyA and Vp by examining our original data in the manner described by Chou and Talalay (5). The dose-effect relationship of both Vp and CyA essentially followed the basic mass-action principle (4). Unfortunately, exclusivity of effects could not be established from the median effect plot. However, given preliminary data that suggest that CyA may act at least in part via an interaction with P-glycoprotein (6) at the level of the membrane (as does Vp), it is likely that CyA and Vp are mutually nonexclusive. In either case, the combination index technique suggests that CyA and Vp are acting synergistically in modulating the multidrug-resistant phenotype.

The combination index for CyA and Vp in the CEM/VLB 1000 line is represented in Fig. 1. In these calculations we have assumed that the interaction between CyA and Vp is mutually exclusive although the extent of synergism would be substantially enhanced were the interaction to be mutually nonexclusive (7) for the reasons mentioned previously. Synergism is indicated at each level of the combination index less than 1.

Clearly, there is no consensus in the literature about the most accurate means of calculating synergy, although it should be remembered that at the clinical level, interactions whether subadditive, additive, or synergistic may improve the therapeutic index of standard cytotoxic drugs while reducing the toxicity of the biochemical modulators under study.

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[1] The abbreviations used are: CyA, cyclosporin A; Vp, verapamil.

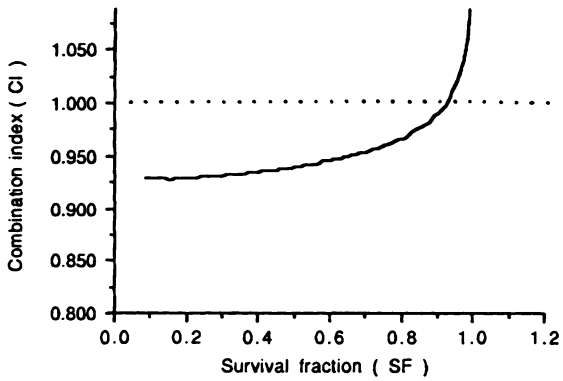


Fig. 1. Median effect plot of combination index compared to survival fraction. The combination index as a function of the survival fraction was derived from the data in Table 3 of the cited paper. CI < 1, CI = 1, and CI > 1, represent synergistic, additive, and antagonistic effects, respectively. In plotting these data, it was assumed that CyA and Vp are mutually exclusive.

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