EDITORIALS

The Search for Cytotoxic Synergy Between Anticancer Agents: a Case of Dorothy and the Ruby Slippers?

William R. Greco, Hélène Faessel, Laurence Levasseur*

In the 1939 movie, "The Wizard of Oz," Dorothy (played by Judy Garland) has many adventures while seeking to return home from the fantasy dream world of Oz. At the end of the movie, she finds out from the good witch of the North that she always had it in her own power to return home by merely clicking together the heels of her magical ruby slippers. The search for synergy, especially with in vitro antiproliferation assays, is reminiscent of Dorothy and the ruby slippers. In some ways, the whole enterprise is like a dream or a fantasy, with thousands of published studies, a myriad of conflicting definitions for interaction terms, many different combined-action assessment procedures, and a wide spectrum of interpretations of similar results. On the other hand, it is often assumed that proper and easy synergy assessment is possible, but that it is necessary for some wizard to tell us the secret.

This editorial will briefly discuss two topics: 1) definition of combined-action terms and 2) interpretation and use of a finding of in vitro synergy. A comprehensive critique of synergy-assessment procedures has been published previously by our group (7). A report in this issue of the Journal by Kaufmann et al. (2) will be used to illustrate specific points.

When two or more entities (people, organizations, drugs, machine parts, etc.) show synergy, by any definition, it is usually considered to be a positive attribute of the combination. In everyday language, synergy is often used to mean "working together in a cooperative, productive manner." The U.S. Patent Office recognizes synergy as one criterion for the necessary characteristic of "unobviousness" for an invention that is a combination of two or more distinct units. Synergistic combinations are thought to be interesting. They are considered special. If you add synergy to a group, you get a team.

Unfortunately, these everyday notions of synergy, which have strong mechanistic connotations, have had a major and often confusing effect on the thinking about drug-drug synergy. Synergy (or antagonism) between two chemical or physical agents is an empirical phenomenon, in which the observed effect of the combination is more (or less) than what would be predicted from good knowledge of the effects of each agent working alone. This general empiric definition of synergy has been interpreted differently by many scientists who have created rival nomenclature schemes and combined-action assessment approaches.

A key difference among the rival schemes and approaches is the choice of the null reference for "no interaction" between the agents. The most common null reference models are the Loewe additivity and the Bliss independence models. Loewe additivity would be observed for a drug combined with itself or perhaps for a drug combined with a very close structural analogue that acts at the same target. For Bliss independence, the fractional response caused by a combination of two agents at specific concentrations is equal to the product of the fractional responses of each agent applied alone at the specific concentrations. Different, independent, mutually nonexclusive sites of action for the two agents are implied. In addition, two of the most commonly used synergy assessment approaches for in vitro studies of combinations of anticancer agents, the method of Steel and Peckham (3) and the method of Chou and Talalay (4), each add an extra null reference model to the mix: the Mode II model (similar, but not the same as Loewe additivity) and the mutually nonexclusive model (similar, but not the same as Bliss independence), respectively. [References (1) and (5) include extensive discussions of terms and definitions.] Also, it should be noted that the validity of the derivation of the mutually nonexclusive model of Chou and Talalay (4) and the main formula used to calculate the combination index for the mutually nonexclusive case have been questioned by our group (7).

Unambiguous links between empirical findings of synergy (or antagonism) and particular cellular or biochemical mechanisms rarely exist. A major problem with assigning mechanisms to findings of synergy is suggested by the work of Jackson (6). Using computer simulations of simple biochemical pathways, he found that by tweaking the values of enzyme kinetic parameters, or by changing the type of enzyme inhibition (competitive, noncompetitive), or by introducing or removing feed-
back from the system, it was possible to generate empiric synergy, antagonism, or "no interaction." The implication is that for complex, highly regulated cellular systems, there will be a plethora of plausible mechanisms for any empiric finding of synergy with two agents. Therefore, without extensive quantitative knowledge of the biochemical pathways targeted by each agent and the connections between those pathways, as well as the capability to conduct computer simulations, most discussions of mechanistic explanations of empiric combined action are mere speculations. Indeed, the extensive discussion of plausible complex mechanisms for the observed empiric antagonisms, synergisms, and shifts from antagonism to synergism found by Kaufmann et al. (2) suggests to us that mechanistic speculations would have been possible for any observed empiric result.

What does in vitro synergy of two anticancer drugs imply about the in vivo efficacy or clinical efficacy of a combination treatment? Therapeutic synergy, in which the best combination treatment for two agents in an in vivo or clinical system is more efficacious than the best treatments using either agent alone, does not necessarily follow from a discovery of in vitro synergy (against tumor cells) for the combination. Therapeutic synergy and in vitro synergy are different concepts. As Kaufmann et al. (2) mention, it is the relative actions of the combination treatment against tumor cells and normal cells (selective toxicity) that are important. If a combination treatment is more selective than either of the single-agent treatments, then therapeutic synergy will result. In fact, if a combination is antagonistic against tumor cells, but even more antagonistic against normal cells, then the combination treatment should be effective in vivo and clinically.

The main rationale for combining anticancer agents (which are also effective individually) in the clinic is that by combining agents with nonoverlapping toxic effects, more total tumor-killing poisons can be applied without increasing the overall toxicity to the host beyond acceptable limits. A second rationale for clinical combination treatments is the inhibition of the development of resistance to either of the single agents. A third rationale is the possible efficacy of combination treatments against heterogeneous tumors that include some tumor cells not inherently resistant to one agent and other tumor cells not inherently resistant to the other agent. A fourth rationale is possible direct empiric synergy of the two agents against tumor cells. It is only this fourth rationale that is routinely examined in our in vitro antiproliferation studies of combinations of agents.

So, if in vitro synergy studies are not directly useful for either elucidating mechanisms or predicting clinical efficacy, what good are they? We do not mean to squelch mechanistic discussions about the combined actions of antineoplastic drugs. Our group has indulged in this activity in the past and plans to indulge even more in the future. Rather, we want to emphasize that mechanistic discussions about combinations should be recognized as more speculative than similar discussions about single agents. Such discussions will become more useful as the details of molecular and biochemical pathways become better understood and the practice of computer-simulating complex biologic systems becomes more widespread. In addition, it may be useful to study the changes observed in a well-characterized, empiric combined action caused by changes in other variables. Other variables could include other drugs, other cell lines, temperature, pH, etc. Hints at possible mechanisms and hints for possible clinical therapies may result. It may be instructive to optimize an empiric in vitro synergy. Furthermore, if and when "normal cell" assays become routine, it may be useful to study combinations of agents in tumor cells and normal cells in parallel and to combine their concentration-effect surfaces to predict selective toxicity and therapeutic synergy. Finally, extreme, unambiguous cases of in vitro antagonism against tumor cells in culture may warrant caution in bringing the combination treatment to the clinic; extreme, unambiguous synergy may warrant enthusiasm for developing the combination treatment further.

The usefulness of assessments of combined action will greatly depend on the validity of the paradigms used for the assessments. Paradigms include definitions of terms and statistical procedures useful for the design of experiments and the analyses of results. A careful and thorough review of different paradigms by the cancer research community is long overdue. The implications of synergy assessment are real and potentially profound. Good synergy assessment and interpretation will never be simple. Clicking our heels together will not provide the answer. The search for synergy is not a case of Dorothy and the ruby slippers.

References