Editorial

Discodermolide: Just Another Microtubule-stabilizing Agent? No! A Lesson in Synergy

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In our increasingly sophisticated world, it is remarkable how, even today, most decisions on combination chemotherapy regimens are still made on purely empirical grounds. Whereas several strategies have been pursued, the most common approach is to combine agents based on their single-agent activity and toxicity profile. Thus, if drug A has 20% activity in disease X as a single agent, and drug B has 20% activity, drugs A and B are combined. An alternate strategy that is increasingly being used is to combine a new agent (gemcitabine, for example) with as many existing agents as possible. Other strategies exist.

Using these empirical approaches, insightful oncologists have crafted successful combinations in a variety of diseases, including malignant lymphomas, testicular cancer, and others (1, 2). To be sure, the road has been bumpy, and success was not always forthcoming. For example, a detour was taken in the treatment of lymphoma, for which combination regimens containing eight drugs were used before returning to simpler regimens (3). Meanwhile, two drugs with highly similar mechanisms of action (cisplatin and cytoxan) were combined in the treatment of ovarian cancer, with arguable improvement in efficacy (4). The virtue of this latter combination may have been to allow one to administer more of this class of active agent with less toxicity. However, it failed to exploit the potential for synergy from combining agents from different drug classes (see below).

An increasing number of attempts are being made to aid this process with preclinical studies, but this is often wanting because: (*a*) clinical development cannot wait for the data; (*b*) there is a reluctance to obtain such data because *in vitro* observations are not always predictive of clinical efficacy; and (*c*) *in vivo* combination studies in animals are difficult to perform, time consuming, and expensive. All too often, the data emerge after clinical trials have been completed. For example, the order of administration of paclitaxel in combination regimens was empirical before preclinical studies demonstrated that additive responses were seen most often when paclitaxel was administered first, with antagonism found in several combinations when paclitaxel was administered second (5).

An "unwritten tenet" has guided most choices: combination regimens should include only one drug from each of the different "classes" of agents (alkylating agents, antimetabolites, antimitotics, anthracyclines, topoisomerase poisons, and so forth). Although this tenet appears reasonable, one can envision some exceptions. For example, the combination of a microtubule depolymerizing agent (an antimitotic) with a microtubule polymerizing agent (another antimitotic) can be synergistic. Although these agents share the same target, they act in different ways. But would one combine two antimitotic agents with a similar mechanism of action? Probably not. However, the study by Martello *et al.* (6) in this issue suggests otherwise.

Martello *et al.* (6) report that Taxol and discodermolide represent a synergistic drug combination. This was a surprise to us and, we suspect, to them . This was a surprise because in may ways, their mechanism of action appears indistinguishable: (*a*) both agents induce polymerization of purified tubulin with and without microtubule-associated proteins or GTP; (*b*) both agents form polymers that are stable to cold and calcium; (*c*) discodermolide competitively inhibits the binding of [^{3}H]paclitaxel to tubulin polymers; and (*d*) both agents cause cell cycle perturbations and induction of a hypodiploid cell population (7, 8). But are Taxol and discodermolide truly indistinguishable? The answer to this is a clear no! To Martello *et al.* (6), the first clue came from the characterization of a Taxol-resistant cell line, A549-T12. Previous studies have shown that A549-T12 cells are not only resistant to Taxol, they are also dependent on Taxol for growth (9). When they examined the cross-resistance profile of this human lung carcinoma subline to various microtubule active agents, they found much less cross-resistance to discodermolide than to Taxol. Furthermore, when they examined the ability of three distinct classes of tubulin-stabilizing agents to substitute for Taxol in sustaining the growth of A549-T12 cells, the differences between discodermolide and the other microtubule-stabilizing agents became even more apparent. Whereas the epothilones and the eleutherobins could substitute for Taxol, discodermolide could not. The observation that followed was possibly expected: in A549-T12 cells, discodermolide was significantly more potent in the presence of Taxol; whereas Taxol did not affect the potency of the epothilones or the eleutherobins. But was this unique to this drug-resistant cell line? Surprisingly, no. Using the combination index method of Chou and Talalay, Martello *et al.* (6) show unequivocally, in four different human carcinoma cell lines, a schedule-independent synergistic interaction between Taxol and discodermolide. This effect was not observed between Taxol and epothilone B.

The challenge now is to understand how this synergy occurs. Martello *et al.* (6) demonstrate that the combination of Taxol and discodermolide at doses that "do not induce mitotic arrest" causes an increase in the hypodiploid population. They conclude this indicates that "a possible mechanism for the observed synergy is the potentiation of apoptosis." However, apoptosis is the end result of something more basic and "upstream" that we do not yet understand. For students of microtubules, this underscores how much more is yet to be learned.

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The fact that we still have much to learn is important because compared with the majority of "molecular targets," our understanding of microtubule structure, function, and drug effects is substantial (10, 11). Unlike textbook pictures demonstrating a beautiful but "static" microtubule spindle in a dividing cell, we recognize that microtubules are intrinsically dynamic polymers, whose dynamic properties are critical for cellular functions. Microtubules grow and shorten by the reversible noncovalent addition and loss of tubulin dimers at their ends. In interphase cells, "cytoskeletal microtubules" exchange their tubulin subunits with soluble cytoplasmic tubulin dimers with half-times of approximately 3 min to several hours (12, 13). As mitosis begins, this cytoplasmic network disappears and gives way to the more dynamic "spindle microtubules" that exchange their tubulin with the soluble pool with half-times of about 15 s (12, 14). This rapid exchange translates to rapid dynamics and is critical for spindle function. In this context, it is probably not surprising that drugs that interfere with microtubule dynamics would be effective against actively dividing cells. It is probably also not surprising that very little binding is required. Thus, whereas at high paclitaxel concentrations, the stoichiometry of paclitaxel binding is 1 mol of paclitaxel/mol of tubulin, much less paclitaxel is required to inhibit tubulin dynamics. At cytotoxic concentrations (10–50 nM), binding of as few as one paclitaxel molecule every 270 tubulin dimers can substantially reduce the rate and extent of shortening at microtubule ends (15). In this case, presumably, microtubule shortening occurs until a paclitaxel molecule is reached, at which point shortening stops. Because of the "low occupancy" of paclitaxel on microtubules, additional drugs could easily be added to microtubules. One would predict that this would translate into additivity for drug combinations using different microtubule-stabilizing agents, and it is this additivity that Martello *et al.* (6) found when Taxol and epothilone B were added together, but not when discodermolide was added to Taxol. With Taxol and discodermolide, unequivocal synergy was observed. Synergy was comparable to that seen when two drugs with distinct mechanisms of action are added together.

How can this be explained? For starters, it seems improbable that this is a result of close proximity of the drugs. With the low "occupancy rates" seen at the concentrations at which synergy is observed, the likelihood of two adjacent tubulin molecules having bound drug is very low. However, it is possible that different tubulin isotypes have differing affinities or that the drugs discriminate among the various forms of microtubules (cytoskeletal, spindle, and microtubule-organizing center). It would have been valuable to have studies examining *in vitro* polymerization to determine whether this synergy occurs with purified tubulin. Alternatively, as Martello *et al.* (6) point out, "the mechanism of synergy may be completely unrelated to the tubulin binding properties of discodermolide, which was originally described in the literature as an immunosuppressant." This implies that discodermolide and/or paclitaxel have additional targets and that these are independent of tubulin binding. In the current context, any such target must be "susceptible" to the low drug concentrations at which synergy occurs. Do such targets exist? Possibly, although there is no clear answer in the literature. For example, phosphorylation of bcl-2 that occurs after administration of paclitaxel and all microtubule active agents may be a consequence of drug-induced cell cycle arrest (16–18). If targets other than microtubules exist, are they targets at low drug concentrations? Possibly, although very low concentrations of paclitaxel have pronounced effects on microtubule dynamics, and this is sufficient to cause cell cycle arrest and cell death (19, 20).

Which, then, is the most likely explanation? We favor a microtubule-based explanation. Our bias is influenced by: (*a*) the data of Martello *et al.* (6) showing synergy between Taxol and discodermolide in four different cell lines (all would have to have very similar secondary targets); (*b*) our own observations that epothilone-resistant cells with β-tubulin mutations are not cross-resistant to discodermolide (similar, yes; identical, no);[2] (*c*) the observation that discodermolide is more potent than paclitaxel in hypernucleating microtubules (potentially different outcomes after binding; Ref. 21), and (*d*) data from the National Cancer Institute drug screen that indicate that microtubule-stabilizing drugs cluster together (the high correlations observed in this analysis suggest that these drugs share a common target, *i.e.,* microtubules, and that this plays a major role in their activity).[3]

Does it matter which explanation is correct? Maybe not. But if synergy is a result of differences in microtubule interactions, then a target that is very attractive to us (tubulin) becomes even more attractive. It raises the possibility that subtle differences among the various microtubule-stabilizing agents may translate into clinical differences in their activity profile. Is there a precedent for this? Yes, the *Vinca* alkaloids. Had scientists given up searching for additional microtubule-depolymerizing agents after vincristine (activity in leukemias/lymphomas) or after vinblastine (activity in testicular cancer), vinorelbine (activity in breast, ovarian, and non-small cell lung cancers) would not have been identified. Is there reason to believe the same will be true of the taxanes, the epothilones, the eleutherobins, the discodermolides, and whatever else follows? If you believe that differences between Taxol and Taxotere exist, then you will not be surprised if we discover that the epothilones, the eleutherobins, and the discodermolides are more than just "other microtubule-stabilizing agents." Nature's greatest strength is its diversity. And if the yew tree (paclitaxel), a soil bacterium (epothilones), and a sponge (discodermolide) cannot provide us diversity, what else can?

Finally, these results demonstrate that potentially synergistic combinations are difficult to predict. It would seem wise to direct greater efforts to study potential combinations early in the development of a drug. With the proliferation of drugs aimed at "novel targets," we might be surprised at how often we are surprised.

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