IN THE SPOTLIGHT

Drug Interactions: The Importance of Looking Inside Cancer Cells

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Summary: *nab*-Paclitaxel increased intratumoral gemcitabine levels by reactive oxygen species–mediated degradation of cytidine deaminase, the rate-limiting enzyme in gemcitabine inactivation. This not only has implications for how this drug combination mediates anticancer effects but also demonstrates the importance of evaluating mechanisms of drug activity within malignant cells. *Cancer Discovery; 2(3);* 208–10. *©2012 AACR.*

Commentary on Frese et al., p. 260 (1).

Most chemotherapy combinations have been developed on the basis of considerations of single-agent activity, nonoverlapping toxicities, and a different mechanism of action that in theory should be additive or synergistic. Evaluations of mechanisms by which agents actually enhance or inhibit each other's activity within malignant cells are uncommon. In this issue of *Cancer Discovery*, Frese and colleagues (1) report that nanoparticle albumin-bound (*nab*)-paclitaxel increased the levels of intratumoral gemcitabine in a mouse model of pancreatic ductal adenocarcinoma (PDA) as the result of a significant decrease in cytidine deaminase (Cda), the primary enzyme that metabolically inactivates gemcitabine. *nab*-Paclitaxel increased reactive oxygen species (ROS), which led to the degradation of Cda without any modulation of mRNA levels. The combination of *nab*-paclitaxel and gemcitabine had significantly greater antitumor activity than either agent alone. Along with evidence from a number of previous studies, this finding argues that part of the reason gemcitabine is not more effective against PDA is because of inadequate drug levels within tumor cells and that approaches that increase these levels should improve efficacy (2). In contrast to what was previously reported with a human tumor xenograft mouse model, there was no detected effect of *nab*-paclitaxel on the stroma, although as the authors point out, there were differences in how the experiments were conducted (3).

The authors are to be congratulated for elucidating a mechanism at the cellular level that might play an important role in the antitumor activity that has been observed with the gemcitabine plus *nab*-paclitaxel combination against PDA (3). Although, as the authors reference, investigators of previous studies had reported increased levels of the most active gemcitabine triphosphate metabolite [2′,2′-difluorodeoxycytidine triphosphate (dFdCTP)] when paclitaxel was administered before gemcitabine, the mechanism for this was unknown. Understanding the mechanism should allow additional studies on optimizing use of these agents in combination. This is especially important, given the 15 years of relative futility in evaluating combinations of gemcitabine with other chemotherapeutic agents in the treatment of PDA. The finding that the enhancement of gemcitabine levels by *nab*-paclitaxel is mediated by ROS adds further caution to the use of supplements that might interfere with this by patients being treated with chemotherapeutic agents when the potential role of ROS in antitumor effectiveness is not known.

These findings also point out the importance of carefully considering which animal model systems to use in addressing specific questions (4). There are strengths and weaknesses of xenograft versus genetic models, but it is likely that the tumor–stromal interaction is more faithfully represented in the genetic model, where the tumor and stromal tissues are of the same species and develop from the initiation of the malignancy. This does not mean that stromal changes do not play a role in *nab*-paclitaxel's enhancement of gemcitabine activity, but further evaluation is needed, and is ongoing in a clinical trial (ClinicalTrials.gov identifier: NCT01442974). The use of an animal model provided adequate tumor tissue at appropriate time points to allow detailed analyses of the tumors for mechanisms of interaction between the 2 agents. This has been lacking from most studies in humans because of the difficulty of obtaining repeat biopsies of malignant lesions, especially with sufficient tissue to perform detailed analyses. Thus, as the authors and others have demonstrated, these are important models for evaluating new treatment approaches (4).

How can this information be used to improve therapy for pancreatic cancer? It turns out that taxanes are not unique among chemotherapeutic agents in decreasing Cda levels. The authors demonstrated that cisplatin has a similar effect, also by the generation of ROS. Although individual trials of combinations of platinum compounds with gemcitabine have not produced significant improvements in survival in PDA, there has been a trend in favor of the combinations (5). Of importance, the combination of cisplatin and gemcitabine produced a survival advantage over gemcitabine alone for biliary cancer and is now the standard approach for unresectable or metastatic disease (6). Reevaluation of how to optimize gemcitabine–platinum compound scheduling is worth considering.

Clearly, a number of factors other than levels of the rate-limiting enzyme (Cda) in inactivating gemcitabine are involved in determining its activity. After phosphorylation, elimination

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Table 1. Examples of current and future directions in treatment of unresectable pancreatic cancer

Current chemotherapy	Phase III trials either ongoing or data pending	Early-stage trials/Future directions
FOLFIRINOX	Gemcitabine ± *nab*-paclitaxel[a]	Correlates of gemcitabine response and resistance[a]
Gemcitabine	Gemcitabine + capecitabine ± GV1001 (telomerase peptide vaccine)	Molecular targets KRAS (targeting downstream pathways/ synthetic lethals) Autophagy Hedgehog inhibitors Metabolism Growth factor receptors Vaccines
Gemcitabine + erlotinib (EGFR inhibitor)	Gemcitabine ± AMG479 (antibody targeting IGF1R)	Biology of circulating tumor cells
Gemcitabine based combinations		Molecular imaging
Capecitabine (or 5-FU and leucovorin)		

Abbreviations: EGFR, epidermal growth factor receptor; FOLFIRINOX, 5-fluorouracil, leucovorin, irinotecan, and oxaliplatin; 5-FU, 5-fluorouracil; IGF1R, insulin-like growth factor 1 receptor..

[a]Studies impacted by this study.

also can occur by deoxycytidylate deaminase, although this accounts for a relatively small percentage. In addition to elimination, a number of processes (uptake; activation; exposure; proteins controlling DNA synthesis, repair, and cell-cycle progression; and epigenetic factors, e.g., microRNA) are critical in determining gemcitabine's activity. Uptake occurs via nucleoside transporters (3). Activation occurs primarily via deoxycitidine kinase (Dck), which phosphorylates gemcitabine to its monophosphate and is the rate-limiting step in the formation of the triphosphate (3). There are also genetic variants of each of these that can affect activity (7). Although it is generally much more difficult to activate protein function than to inhibit it, approaches aimed at increasing activity of nucleotide transporters or Dck in tumor cells could be considered.

The most straightforward approach to modify exposure is via prolonged gemcitabine delivery by dose rate infusion (DRI). Preclinical data suggest that prolonged exposure of cancer cells to gemcitabine enhances the killing of cells (3). Unfortunately, although preliminary studies suggested a benefit for DRI gemcitabine, a phase III trial in patients with PDA did not show significant improvement compared with gemcitabine delivered at the standard dose schedule (3, 8). However, differences in exposure might have not achieved sufficiently increased dFdCTP levels within malignant cells as compared with normal cells to provide a therapeutic advantage. Although levels of the active metabolite in peripheral blood cells increased with DRI as compared with standard infusions, direct comparisons in tumor tissue have been limited (3). The evaluation of whether there is a differential increase in tumor versus normal tissues in a genetic animal model might provide additional insight into whether this approach is worth reconsidering, perhaps with different dose schedules or combinations with other agents. Evidence also exists that the pharmacologic advantage of DRI might only occur for a subset of Cda alleles, which may limit its general applicability, although this finding needs additional study (9).

Modulations of the function of proteins involved in DNA synthesis and repair or cell-cycle control are other potential mechanisms to enhance gemcitabine activity (3). Epigenetic approaches, such as manipulation of various microRNAs, might also modulate gemcitabine function, although considerable work is needed before this may be achievable in patients (10). Targeting several of these different factors impacting gemcitabine function is more likely to be effective than any single approach. However, there remains the fundamental issue for the idea of increasing gemcitabine levels in malignant cells that it is not sufficient to increase active drug levels in tumor tissue if levels are being increased to the same extent in normal tissues with no resultant improvement in therapeutic index. This will have to be carefully evaluated for any of these approaches. Perhaps the concentration of *nab*-paclitaxel in PDA may help provide this advantage, but this is yet to be determined (3).

The phase III trial of gemcitabine plus *nab*-paclitaxel versus gemcitabine for PDA is well underway. Its results are awaited to answer the question of whether this will be a therapeutic step forward and join FOLFIRINOX (5-fluorouracil, leucovorin, irinotecan, and oxaliplatin) as potential options for patients with PDA who have good performance status (refs. 3, 11; Table 1). The hope is that gemcitabine plus *nab*-paclitaxel will also have less overall toxicity than FOLFIRINOX, although this awaits the results of the ongoing phase III study (3). Continued evolution of the understanding of the biology of pancreatic cancer has provided a number of targets being explored, alone and in

combinations with each other and chemotherapy (Table 1). Frese and colleagues (1) in their article point out the importance of continued evaluation of preclinical therapeutics in appropriate models to help guide the best approaches to take into human clinical trials as well as understanding mechanisms of activity to make improvements.

To complement this, where possible and when it can be safely done, the use of tumor biopsies in human clinical trials to define mechanisms of response and resistance needs to be considered. A clinical trial in which investigators evaluate factors important in intratumoral gemcitabine levels is ongoing and should provide additional insight (ClinicalTrials.gov identifier: NCT01276613). Increased understanding of the biology of circulating tumor cells may make these an attractive alternative to the need for repeat biopsies, although additional work is still required. As methods for molecular imaging *in vivo* improve, it is our hope that these will allow evaluations of drugs, protein levels, and other molecules inside tumor cells that currently require tumor biopsies.

In terms of specifically applying information learned from this study, could the determination of intratumoral Cda protein levels or genetic variants with different function help guide which patients are more likely to respond or have increased toxicity from gemcitabine? Measurements of Cda levels, as well as levels of other proteins and genes important for gemcitabine activity, such as Dck, are feasible from clinical samples and need further evaluation in terms of their ability to predict efficacy and toxicity. Most importantly, there needs to be continued close collaboration in both directions between the clinic and laboratory to increase understanding of biologic processes to help guide approaches for optimizing cancer therapy.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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