## *Editorial*

# Overcoming Methodological Weakness in Dose-Intense Alkylating Agent Studies through Pharmacology

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The importance of alkylating-agent dose intensity to cancer treatment is the subject of considerable comment. In recent years, this debate has often centered on the value of hematopoietic progenitor cell-supported high-dose alkylating agent regimens used in the treatment of hematopoietic cancers, Hodgkin's disease, and breast cancer. Large randomized trials, lasting up to 10 years and requiring hundreds of patients, are usually mounted to answer these questions. The barriers to the execution of these trials are formidable. When results are inconsistent or negative, guides to treatment and future research are often obscured. Although randomized trials are highly desirable to confirm promising Phase II dose-intensity data, the explosion of new therapies, combined with the fact that less than 5% of cancer patients in the United States enroll in clinical studies of all types, virtually guarantees that most dose-intensity questions will never be tested in Phase III trials. Overcoming this deficit will require a "sea change" in patient participation in clinical trials or the development and use of alternative methods to define the clinical benefit of dose-intensity approaches. This is particularly true because doses may be varied infinitely, the impact of heightened toxicities may bias patient enrollment, and the complexity and cost of hematopoietic cell transplantation reduces the availability of such trials to patients. Can nonrandomized trials, combined with insightful pharmacological studies, refine our view of the value of dose-intensity in cancer and direct Phase III strategies?

In this issue of Clinical Cancer Research, Yule *et al.* (1) report sophisticated pharmacological studies that strongly support the concept that higher exposure to cyclophosphamide (CPA) reactive intermediates improved the relapse-free survival of children with non-Hodgkin's lymphoma. CPA is a prodrug that must be activated by hepatic microsomes, particularly cytochrome P-450 3A4 (P450 3A4; Ref. 2). Phosphoramide mustard (PAM), produced by this bioactivation, is primarily responsible for the cytotoxic effects of CPA. Fig. 1 summarizes, in abbreviated manner, the critical components of CPA metabolism.

CPA is hydroxylated by microsomes to produce 4-hydroxyCPA, which exists in chemical (nonenzymatic) equilibrium with its aldehyde tautomer, aldophosphamide. Aldophosphamide undergoes nonenzymatic conversion to PAM as well as being enzymatically converted to inactive products. Dechlor-

ethylation also deactivates CPA. Thus, CPA bioactivation, dechlorethylation, and reactive intermediate elimination are governed by variable biological processes, and the remaining important components of CPA disposition are nonenzymatic and minimally variable. On the basis of these principles, processes that produce more rapid CPA activation and, thus, lower plasma CPA area under the curve (AUC) will increase PAM exposure. In a similar fashion, processes that increase the rate of CPA reactive intermediate elimination will decrease PAM exposure (Table 1). By measuring CPA, carboxyphosphamide, and dechlorethylCPA, Yule *et al.* (1) convincingly demonstrate that pediatric lymphoma patients experiencing increased PAM exposure had superior treatment outcome.

Two important conclusions can be drawn from this study.

(*a*) CPA reactive intermediate exposure is a critical determinant of treatment outcome. Within the range experienced by patients in this study, more is better.

(*b*) CPA metabolic activation and reactive intermediate metabolic elimination both vary from patient to patient. This variation produces a range of PAM exposures that is not directly predicted by the delivered dose. This variability is determined by the net variation in CPA metabolic pathways between patients.

This patient-to patient variability in reactive intermediate exposure may be produced by genetic differences in enzyme activity (3), drug transport activity (4), inducers or inhibitors of the relevant metabolic pathways (5), or transcriptional or translational alterations. Although the variable patient outcome produced by these effects is at first glance undesirable, it offers an opportunity to directly probe the relationship between alkylating-agent reactive intermediate exposure and patient outcome. In a relatively small Phase II trial, detecting a strong correlation between increases in reactive intermediate exposure and favorable outcome provides greater observational power than historical comparison of clinical outcome from older trials. Such small pharmacological hypothesis-generating translational studies can allow better selection of targets for Phase III dose-intensity trials and perhaps more effective design of the Phase III studies themselves.

In addition, the study by Yule *et al.* emphasizes the intrinsic weakness of trials that aim to test the value of dose intensity by delivering two different "standardized" doses of alkylating agents to patients by randomization. In fact, the cohorts in these trials will often be quite heterogeneous in reactive intermediate exposure because of the factors just described. Such studies will usually have considerably less power to detect the effect of differences in drug exposure than that expected when the delivered dose is naively used as a dichotomous independent variable for design and analysis.

The activity of alkylating-agent bioactivation and elimination pathways is becoming increasingly predictable through the use of model compound metabolic studies [*e.g.,* erythromycin breath testing to measure P450 activity (6)], small "pilot" dose
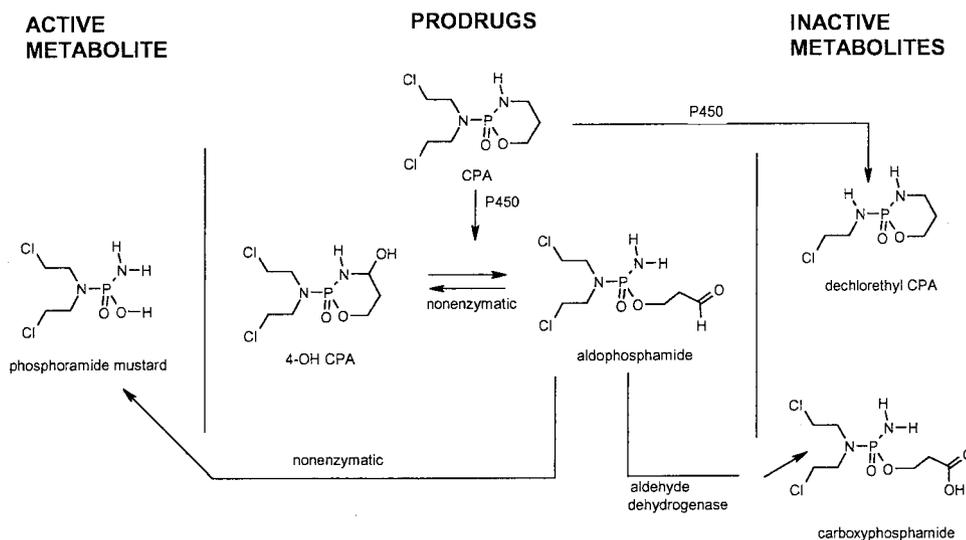
*Fig. 1* Critical components of cyclophosphamide (CPA) metabolism. *P450,* cytochrome P-450.

pharmacokinetic studies (7), detection of enzyme genetic polymorphisms (4), and therapeutic drug monitoring (8). Although these studies add technical complexity, they should be considered as important to dose-intensity studies as the measurement of specific molecular targets is to the evaluation of the new generation of targeted therapeutics. Absent such correlative studies, even large randomized trials that test dose intensity may produce false conclusions simply because the delivered dose of parent drug is only loosely correlated to the delivered therapeutic potency of the reactive intermediate(s). These studies are of equivalent importance in understanding toxic outcome and the correlation of dose intensity to therapeutic index (9). When "definitive" randomized trials of alkylating-agent dose intensity are designed without careful attention to dose-related pharmacological issues, conflict between trials or "negative" results should not be surprising, nor should extensive variability of toxic effects. Pharmacological analysis should become a central

component in trials in which dose-effect conclusions are a primary objective.

## Acknowledgments

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*Table 1* Effect of enzyme activity on cyclophosphamide (CPA) pharmacology

When the 4-position of the oxazaphosphorine ring is oxidized more rapidly, there is increased production of phosphamide mustard (PAM) and greater antitumor effect. With increased oxidation of the mustard sidechains or increased oxidation of aldophosphamide, PAM production is decreased, producing less antitumor effect. Up arrows indicate an increase in activity or concentration, down arrows indicate the reverse, and dash indicates no net change.

| | Metabolite | | | |
|---|---|---|---|---|
| Metabolic change | CPA | PAM | DCE CPA[a] | Carboxy PA |
| ↑ Ring oxidation | ⇓ | ⇑⇑ | ↓ | — |
| ↑ Sidechain oxidation | ↓ | ↓ | ↑ | — |
| ↑ Aldehyde dehydrogenase | — | ↓ | — | ↑ |

[a] DCE CPA, dechlorethyl cyclophosphamide; Carboxy PA, carboxyphosphamide.

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