Reply to Koh: Signaling dynamics of DNA damage response invoked by combination therapy are dose-dependent

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Koh (1) correctly notes that gemcitabine elicits two mechanisms of action, but proposes that chain termination predominates. We believe its ability to inhibit ribonucleotide reductase and deplete dNTP is more critical (2). We have previously discussed our rationale for this position (3). Both mechanisms result in S phase arrest and then replication catastrophe upon addition of a CHK1 inhibitor. Koh agrees with this conclusion (4). His primary concern is that we use high concentrations of gemcitabine.

Our experiments are designed to reflect clinically relevant concentrations and schedules. Patients receive gemcitabine by a short infusion, and it is cleared rapidly from plasma. Accordingly, we incubate cells briefly with gemcitabine. After removal, cells continue to accumulate in S phase as observed *in vitro*, xenografts, and human tumors (3). The CHK1 inhibitor is most effective when administered after cells arrest in S phase (5). In contrast, Koh incubated cells with gemcitabine and CHK1 inhibitor continuously for 72 h (4), neither of which is a clinically relevant schedule. Koh notes his low concentrations of gemcitabine permit continued proliferation for 72 h (4). These low concentrations are not clinically relevant as the standard dose (1000 mg/m^2) causes durable S phase arrest in the tumor cells (3). We also showed that considerably lower concentrations of gemcitabine in xenograft models exhibited durable S phase arrest (3). Consequently, low concentrations of gemcitabine that permit ongoing replication have little relevance to the clinical administration of these drugs. Consequently, we believe our experimental design is far more relevant to human patients.

The authors declare that they have no conflicts of interest with the contents of this article.

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