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Commentary

It takes two to tango, and the right music: Synergistic drug combinations with cell-cycle phase-dependent sensitivities

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The basic principle of cancer therapy is the specific killing of cancer cells while sparing normal cells and tissues from the detrimental effects of treatment. Since the application of anticancer drugs, including "classic chemotherapy", the importance of "cycling kinetics" have been recognized. The therapeutic window of cancer therapy is in a large part attributed to the observation that proliferating cells display higher sensitivity than nonproliferating cells. More than three decades ago, a distinction was already made between "cycle-specific" and "cell cycle stage-specific" drugs. It was argued that such distinction could provide the means to avoid increased toxicity associated with combination chemotherapy without loss of therapeutic effects [2]. In addition, around that time it was also shown that cell cycle synchronization by pre-treatment with hydroxyurea, a DNA synthesis blocker, could increase the effects of cytostatic drugs like vincristine, predominantly acting in S-phase cells in an *in vivo* model [9].

In recent years, the repertoire of cancer drug targets has further expanded with components of deregulated pathways in cancer such as cell cycle control, DNA damage, replication stress, aberrant metabolic activity and proteotoxic stress. For many of these processes, it is expected that cancer cells display different sensitivities not only determined by their proliferation rate but also by specific phases in the cell-cycle.

It has become clear that single drug treatments are limited in their effectiveness. In most cases clinical responses are short-lived, patients' tumors rapidly develop resistance, leading to recurrence with modest effects on overall survival. As a consequence, it is thought that combinations of drugs are needed to overcome resistance and increase the overall clinical benefit. An important consequence of cell cycle phase-specific sensitivity is that combinations of drugs can be synergistic or antagonistic, where one drug prevents the progression of the cell cycle to a phase where the second drug has its effect. An example is the antagonistic combination of cisplatin, preventing the progression of the cell cycle and paclitaxel, acting in mitosis by interference with the assembly of the mitotic spindle. For the further development of effective cancer drug combinations, it is important to understand their cell cycle phase dependency, how this affects their interactions, and how this can potentially be used for the selection of the most effective synergistic combinations.

Recently reported in *EBioMedicine*, Johnson et al. describe a cell cycle synchronization method combined with high throughput measurements of drug effectiveness in different cell cycle phases [3]. By using the reversible CDK1 inhibitor RO-3306, they were able to define two 6 h time windows in which cells can be exposed to drugs reflecting G1- or S/G2 phases of the cell cycle. Using their experimental method, they established dose-response curves for G1- or S/G2-specific exposure for a collection of more than 200 cancer drugs. From these dose-response curves they calculated a cell cycle specificity score (CCS) for each compound with a score <0 indicating G1 sensitivity and >0 S/G2 sensitivity. They observed cell cycle phase dependencies for a large proportion of drugs tested. For those treatments known to be associated with specific cell cycle phases such as chemotherapy and DNA damage checkpoint inhibitors, they observe CCS scores indicating S/G2 specificity. Compounds associated with specific pathways such as MAPK and PI3K show CCS scores for G1 specificity. It is important to note that the potency of the drugs was reduced when comparing a 6 h pulse versus continuous treatment. Surprisingly, in some cases, compounds with the same target yielded opposite CCS scores despite having equal potency. The reason for this discrepancy is not known but could indicate differences in drug pharmacokinetics and off-target effects for drugs with the same target.

Based on the observation of cell cycle-specific sensitivities for single drugs, one could argue that a combination of two drugs with a similar CCS score could result in synergistic effects. Indeed, for previously identified synergistic combinations of a CHK1 inhibitor (AZD7762) or an ATR inhibitor (AZD6738) combined with DNA damaging agents like gemcitabine, CCS scores indicate high S/G2 specificity for each drug of the combination [4,10]. To explore whether a synergistic combination of drugs could also show cell-cycle phase dependent sensitivity, Johnson et al. investigated the combination of the ATR inhibitor AZD6738 and gemcitabine, both having S/G2 specificity. To mimic an *in vivo* dosing schedule, the authors applied a continuous low dose of AZ6738 following a 6 h pulse of gemcitabine in G1- or S/G2-accumulated cells. Surprisingly, the concentration of gemcitabine required for 50% growth inhibition was reduced in G1-

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enriched cells. This suggest that this combination is more effective in G1-accumulated cells, although both drugs have S/G2-specific mechanisms. However, due to the set-up of this experiment, the enhanced cytotoxicity can also be explained by cell cycle synchronization with the entire cell population progressing synchronously through the following S-phase. To test whether transient G1 accumulation followed by a release also enhances the effects of the gemcitabine/AZD6738 combination, they used palbociclib, a reversible CDK4/6 inhibitor already in clinical use [7]. Using a "clonocidal assay", in which cells are first allowed to form colonies, after which they are arrested in G1 with palbociclib and subsequently released while being exposed to the different drugs or their combinations. They reported that cell populations synchronously entering S-phase are more sensitive to the synergistic combination of S/G2-specific drugs gemcitabine and AZD6738.

The experiments by Johnson et al. using a CDK4/6 inhibitor show that cell cycle synchronization can enhance drug sensitivity. However, sequential drug treatments are not restricted to cell cycle synchronization. Insights in dynamic rewiring of signalling pathways, induced cell-state changes or even acquired vulnerabilities due to an initial drug treatment have also provided rationale for the sequential application of cancer drugs. Examples of such strategy are the pretreatment of triple negative breast cancer cells with EGFR inhibitors which markedly increases their apoptotic response to chemotherapy [6] and the acquired vulnerability of therapy resistant cancer cells to GPX4 inhibition as result of a mesenchymal to epithelial transition [8]. Another interesting strategy is to block cell cycle progression, for example by using CDK4/6 inhibitors, only in normal (stem) cells and not in Retinoblastoma (RB)-deficient tumor cells, to prevent the detrimental effects of treatment with cytotoxic chemotherapy [1].

To further explore the utility of the CCS scores for sequential drug treatments, Johnson et al. used drug response data from a study by Koplev et al. [5] in which they determined a synergy score for thousands of different drug schedules. In this set-up, cells were first treated with drug A for 24 h, followed by drug B for 24 h after which the drugs' effects were measured. Although no differences in CCS scores for drug A were observed in either antagonistic or synergistic schedules, the analysis showed that when an S/G2-specific compound is used as the second drug, it is more likely to be antagonistic. Similar to the observations made by Koplev et al., antagonistic drug combinations were associated with tubulin modulators as second drug treatment. In cases where synergy was observed with this class of drugs they were used as the first compound in the sequence. Although the analysis of this data-set did not provide a clear association between CCS scores and synergy, it could have applications in the prevention of drug scheduling antagonism.

Understanding the interplay between the cell cycle and sensitivity to cancer drugs will provide further insights in their application and for the development of effective drug combinations. The work by Johnson et al. provides a useful platform to determine the cell cycle phase sensitivity of a large numbers of drugs. By combining this platform with different treatment schedules, including sequential and combined drug treatments, will allow a platform to further explore the relationship between cell cycle phase sensitivity and synergistic or antagonistic drug combinations.

Contributors

CL and RLB co-wrote this commissioned Commentary.

Declaration of Competing Interest

The authors declare no conflicts of interest.

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