

## IN THE SPOTLIGHT

## RADical Response Puts an Exceptional Responder in CHKmate: A Synthetic Lethal Curative Response to DNA-Damaging Chemotherapy?

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**Summary:** In this issue of *Cancer Discovery*, Al-Ahmadie and colleagues identify a somatic mutation in the *RAD50* gene as a likely contributing factor to an unusual curative response to systemic combination therapy employing the DNA-damaging agent irinotecan and a checkpoint kinase 1 inhibitor in a patient with recurrent, metastatic small-cell cancer. This study highlights the importance of in-depth analysis of exceptional responders to chemotherapy and targeted therapy in early-phase clinical trials and opens new avenues for developing cancer genome-based combination therapy to improve the efficacy of traditional chemotherapy through synthetically lethal interactions. *Cancer Discov*; 4(9); 988–90. ©2014 AACR.

See related article by Al-Ahmadie et al., p. 1014 (2).

Clinical trials play a critical role in translating the cutting-edge discoveries of cancer research from bench to bedside. The criteria to evaluate the clinical success of candidate drugs are based on the frequency of disease regression or prolonged median progression-free survival. Drugs that fail to induce these desired clinical outcomes in a significant number of patients are often withdrawn from further clinical evaluation. However, the heterogeneous treatment response in patients has long been recognized in clinical trials. In the same clinical trial, some patients can exhibit exquisite sensitivity and/or durable responses to anticancer treatment, and are referred to as “exceptional responders,” whereas others show no clinical benefit and display progression of disease (1). It typically remains unexplored why a subgroup of patients have outlier responses in what are otherwise considered failed clinical trials. However, investigating the molecular markers and mechanisms associated with these exceptional responses provides the potential to revitalize drugs that failed in clinical trials for the majority of patients, but would benefit an identifiable subgroup of patients. Further, these studies have the potential to contribute to the identification of rational drug combinations that can extend the utility of chemotherapies and targeted therapeutics. Thus, it is of critical theoretical and translational importance to identify mechanisms underlying exceptional responders.

Although it remains a clinical challenge to attain curative therapeutic response in patients with metastatic solid tumors,

particularly in early-phase clinical trials, a 51-year-old woman with recurrent, metastatic small-cell cancer achieved a complete and durable response in a phase I clinical trial of AZD7762, an ATP-competitive checkpoint kinase (CHK1/2) inhibitor, in combination with irinotecan, a topoisomerase I (Topo I) inhibitor. To investigate the genetic basis of this outlier example of curative systemic cancer therapy, Al-Ahmadie and colleagues (2) performed whole-genome sequencing of tumor samples and identified a *RAD50*<sup>L1237F</sup> mutation as a potential contributor to the curative response. *RAD50* is a component of the multifunctional protein complex MRN (MRE11–*RAD50*–NBS1) that detects DNA double-strand breaks (DSB), activates the ataxia telangiectasia mutated (ATM) checkpoint kinase, and promotes DSB repair (Fig. 1). The *RAD50*<sup>L1237F</sup> mutation is located in the D-loop of the ATPase domain near the C-terminus of *RAD50*, which, based on studies from the team, seems to be a hypomorphic mutation, with the gene product retaining residual function. The authors found that the impaired function of the MRN complex due to this mutation led to a synthetic lethality in cancer cells when they were treated with CHK1 inhibition and the DNA-damaging chemotherapy irinotecan (Fig. 1).

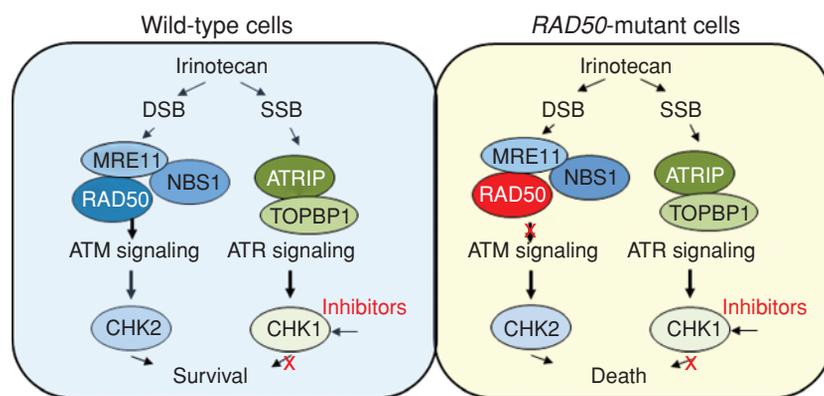
As tumors can harbor thousands of mutations, it is technically challenging to sort out the most relevant mutations of potential functional and biologic significance in exceptional responders to chemotherapy. Al-Ahmadie and colleagues (2) provide valuable insights into delineating tumor genome sequencing data. First, they sequenced the recurrent tumor specimen obtained after standard chemotherapy, but before trial enrollment. They then confirmed the mutations in the diagnostic tumor samples collected before chemotherapy. By comparing treated and treatment-naïve tumors, the authors selected mutations that arose early in molecular time, which were more likely to exert biologic driving effects on tumor development. Second, they performed an integrated analysis using the mutation, DNA copy number, and tumor clonality data with pathway analysis relevant to the mechanism of drug action to prioritize genomic aberrations. Third,

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**Figure 1.** The impaired function of the MRN complex due to a somatic mutation of *RAD50* leads to a synthetic lethality in cancer cells when they are treated with CHK1 inhibitors and DNA-damaging chemotherapy irinotecan.

considering heterogeneity of tumor cells, they confirmed whether the mutation was a prevalent mutation and found that the *RAD50*<sup>L1237F</sup> mutation was detected in the majority of tumor cells. Fourth, they analyzed protein structure to identify the potential functional impact of mutations in the tumor. Finally, the authors took advantage of the evolutionary conservation of *RAD50* function by efficiently modeling its mutations in the *S. cerevisiae* model system, and then recapitulated the findings in mammalian cells.

Mechanistically, irinotecan inhibits Topo I by reversibly abolishing the religation activity of Topo I to generate single-strand DNA breaks (SSB). When replication forks collide with the SSBs, the stalled and collapsed replication forks result in formation of DSBs. Thus, irinotecan-induced DSBs are largely replication dependent (3). In wild-type cells, the presence of SSBs and DSBs activates both ATM and Rad3-related (ATR) kinase-mediated signaling pathways, which enable cells to survive DNA damage by coordinating DNA-damage response (DDR), the cell-cycle checkpoint, and DNA repair (4). Although extensive cross-talk and overlapping functions have been found between these two pathways, in general, activation of ATM is largely dependent on the proper function of the MRN complex in response to DSBs, which in turn activates CHK2. In response to SSB, ATR activation is facilitated by the proteins ATRIP and TOPBP1, which regulate the downstream checkpoint protein CHK1 (4). In contrast, in *RAD50*-mutant tumor cells, the ATM-dependent signaling pathway is compromised due to impaired function of the MRN complex. Thus, inhibition of CHK1 further blocks the ATR signaling pathway, which can lead to a deleterious effect on cell survival. This synthetic lethal interaction in *RAD50*-deficient cells likely explains the exceptional response observed in this patient with a *RAD50* mutation. In contrast, wild-type cells can cope with CHK1 inhibition due to functional compensation from the ATM signaling pathway. Functional redundancy between the ATM and ATR signaling transduction pathways also potentially explains why the majority of patients in this clinical trial did not show therapeutic benefits from the CHK1 inhibitor and irinotecan combination. Although the authors provide compelling data demonstrating that irinotecan and CHK1 inhibition generates a synthetic lethal response in cells with *RAD50* defects, including the specific aberration identified in this patient's tumor, the results remain an association without direct proof. The cogent hypothesis developed from this

single exceptional responder is sufficient to warrant exploration in clinical trials based on multiplex genomic testing for defects in *RAD50* and potentially the MRN complex; however, due to the paucity of *RAD50* aberrations in any single cancer lineage, this will require a concerted pan-cancer community effort combined with comprehensive genomic testing of all patients eligible for clinical trials.

Previous studies of exceptional responders have identified response biomarkers for targeted therapy, such as activating mutations of *mTOR* and *TSC1* mutations for the mTOR inhibitor everolimus or *EGFR* mutations for the EGFR inhibitor gefitinib (5–9). In these studies, DNA-sequencing approaches have been successfully used as a valuable tool to explain the mechanisms underlying exceptional responses to targeted therapeutics and to these resulting hypotheses by studies matching targeted therapy with actionable molecular targets. Compared with these studies, Al-Ahmadie and colleagues (2) demonstrated the impact of extensive genetic analysis on an unusual response to traditional DNA-damaging chemotherapy, although in the current lexicon it is indeed likely that a Topo I inhibitor would be classified as a targeted therapy. Rather than targeting the mutation itself, the study identifies a mechanism-based combination therapy to target the therapeutic vulnerability rendered by the genetic mutation, a compelling example of synthetic lethality. Thus, this study provides us with a new conceptual framework to develop systematic combination therapy through genetic and chemical drug-induced synthetically lethal interaction, which is especially important for optimizing responses to standard DNA-damaging treatments.

In patients receiving radiation therapy and conventional chemotherapy, DNA damage causes cell-cycle arrest and cell death either directly or following DNA replication during the S phase of the cell cycle, in which cell death can be induced when cells attempt to replicate damaged DNA. Thus, DNA-damaging treatments are more toxic to replicating cells than to nonreplicating cells. The therapeutic window for DNA-damaging treatments is offered, in part, by more rapid growth of cancer cells than their normal counterparts. However, a lack of robustness, decreased cell survival signals, or a loss of alternative DNA repair pathways due to the underlying genomic instability of tumors may also be key components. As exemplified by the study by Al-Ahmadie and colleagues (2), the efficacy of DNA-damaging treatment can be modulated by genetic alterations in DDR and DNA repair pathways in cancer cells or,

alternatively, by chemical inhibitors of these pathways, providing an approach to generalize responsiveness in patients with intact DDR and DNA repair pathways. DDR and DNA repair networks are composed of many parallel pathways to maintain genomic integrity, which have both distinct and overlapping functions such as ATM and ATR signaling pathways. As these constitute a barrier of tumorigenesis, genome maintenance mechanisms are often breached by cancer cells through alterations of components of DDR and repair genes during tumor development. These genetic changes themselves may not be actionable targets (e.g., the *RAD50* mutation in this study). However, they can render cancer cells reliant on a reduced set of DDR and DNA repair pathways for survival in the presence of DNA-damaging treatments, which can be subsequently targeted by chemical inhibitors such as CHK1 inhibitor in this case, or potentially an ATR inhibitor, to enhance treatment efficacy. Through a synthetic lethal interaction, the combination of genetic alteration and chemical inhibition of the DDR and DNA repair pathways may transform traditionally DNA-damaging treatments that are not targeted to specific oncogenes in a patient's tumor into new therapeutic regimens, which are highly effective and selective for a specific subgroup of patients.

#### Disclosure of Potential Conflicts of Interest

G.B. Mills has received commercial research support from Glaxo-SmithKline and AstraZeneca; has ownership interest in Catena Pharmaceuticals, PTV Ventures, and Spindle Top Ventures; and is a consultant/advisory board member of AstraZeneca, Blend, Critical Outcome Technologies, HanAl Bio Korea, Illumina, Nuevolution, Pfizer, Provista Diagnostics, Roche, Signalchem Lifesciences,

Symphogen, and Tau Therapeutics. No potential conflicts of interest were disclosed by the other authors.

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# CANCER DISCOVERY

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